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The Metabolism of Normal and Tumor Tissue

XX. A Comparison of the Metabolism of Tumors of Liver and Skin with That of the Tissue of Origin*†

F. Dickens, Ph.D., and H. Weil-Malherbe, M.D.

(From the Research Laboratory, North of England Council of the British Empire Cancer Campaign, Royal Victoria Infirmary, Newcastle upon Tyne, England)

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INTRODUCTION

Since the discovery by Warburg (60) of the glycolytic metabolism of tumors, and the observation by Dickens and Šimer (11, 13) of a lowered respiratory quotient in tumors, it has widely been assumed that they possess a metabolism differing from normal tissue, inasmuch as they combine high aerobic and anaerobic glycolysis with an R.Q. below unity.

Doubt on the significance of the aerobic glycolysis in tumors first arose when Murphy and Hawkins (44), and later Crabtree (8), found that certain new growths of the mouse had little or no aerobic glycolysis. Thus aerobic glycolysis is not invariably associated with neoplasia.

At first it was thought that with certain exceptions, which could be explained on the basis of damage caused by the conditions of measurement, normal and particularly "stationary" tissues did not glycolyze aerobically to any appreciable extent. Later, however, it was found that there are some few normal tissues which glycolyze aerobically to an extent comparable with that of the "typical" transplanted animal tumors, and therefore are much more glycolytically active than those neoplasms having little aerobic glycolysis. The retina was early recognized as such a tissue (62). Later, the medulla of the kidney, unlike its cortex, was found (28, 14) to have a glycolytic metabolism comparable in activity with the most vigorous type of tumor metabolism, and contrary to the observations of György, Keller, and Brehme (28) we found (14) that the same metabolism was shown even in the serum of the same species; evidence that this was not a case of damage due to unphysiological medium, such as is known to occur with some other tissues. We concluded (14) that this tissue—and also cartilage (15)—probably has energy requirements too great for its respiration alone to supply.

In these examples the R.Q., according to our measurements (11, 13, 14), was unity. Bywaters (7), however, found that synovial membrane glycolyzes aerobically and has a low R.Q. Its metabolic activity was of a low order, and a better example of a normal tissue with a "tumor-like" metabolism was found by us (16) in intestinal mucosa; this tissue, from the jejunum of the rat, had a glycolytic and respiratory activity comparable with that of the most vigorously growing neoplasms combined with an R.Q. of about 0.85; its aerobic glycolysis was almost equal to its anaerobic glycolysis. Evidence was presented that mechanical damage could not be held responsible.

The suggestion has therefore been advanced (3) that the high aerobic glycolysis and low R.Q. possessed by most tumors are not in reality a result of any alteration in metabolism occurring during carcinogenesis, but are referable to the fact these growths arose from normal tissues that had the same type of metabolism. This hypothesis has the merit of emphasizing the need for comparison with the tissue of origin, but unfortunately this strict comparison is possible in only a few instances since the cells of origin cannot usually be obtained in a state of purity, even when their nature is known with certainty. Further, we shall show in the Discussion that such a comparison ought to include if possible not only the adult tissue and its tumor, but also the primitive cell which may be regarded as the common ancestor of both.

Since the greatest change is to be expected when the malignant transformation is complete, it is important that the comparison with malignant as well as with nonmalignant tumors should be included in any proper investigation of this hypothesis. We be-

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† Because of the difficulties of international communication the authors have not read proof of this article.

lieve that this point has been overlooked by Berenblum and his associates (2), who based their entire hypothesis on a single comparison between normal skin epithelium and a Shope papilloma, both of which they found to have substantially the same metabolism: No indication was given whether this papilloma had become malignant. This is a fundamental point, because it will be shown in the present paper that, while a benign tumor may have a metabolism almost identical with that of its tissue of origin, a change undoubtedly occurs in those examples studied by us when the tumor becomes malignant.

There appear to be only two other cases besides skin in which the metabolism of the tumor and that of its tissue of origin have already been directly compared. Dreyfuss (17) reported, without drawing any particular attention to the fact, an excellent example—that of human endometrium in various phases and pathological states, compared with uterine carcinoma. His results are summarized in Table I.

TABLE I: METABOLISM OF HUMAN ENDOMETRIUM, AFTER DREYFUSS (17)

	$-Q_{O_2}$	$Q_G^{O_2}$	$Q_G^{N_2}$
Proliferative phase	16.9	1.4	9.7
Secretory phase	16.3	1.0	8.5
Decidua	16.4	0.8	14.2
Hyperplasia of endometrium	16.5	1.1	11.2
Adenomyosis *	16.5	1.2	10.6
Carcinoma of endometrium	13.3	10.0	14.2

* "The term adenomyosis of the uterus signifies the invasion of the myometrium by endometrial glands and endometrial stroma. . . . . Despite this aggressive behavior, adenomyosis is clinically a benign lesion; it never produces metastases and is never fatal. . . . . Its position is thus intermediate between benign and malignant growth processes."

The second example is the group of liver tumors induced by feeding azo dyestuffs to rats. Hayashi and Tomita (30) found that in rats fed aminoazotoluene the formation of adenomas and hepatomas was accompanied by a sudden increase in anaerobic glycolysis. Nakatani and his group (46), who, like all succeeding authors, including ourselves, used *p*-dimethylaminoazobenzene to produce neoplasms, found a gradual increase in anaerobic glycolysis during the development of the tumors; when the period corresponding roughly with malignancy was reached the aerobic glycolysis also increased notably. The remainder of the liver retained the metabolism of the precancerous phase of the dye feeding, *i.e.*, low aerobic with high anaerobic glycolysis. Orr and Stickland (50) could not observe the latter increase of anaerobic glycolysis associated with the precancerous phase; they made the surprising observation that there was no evidence of an increase in the rate of anaerobic glycolysis of liver tissue of rats at any stage of dye feeding. Some of the normal rat livers used by Orr and Stickland had high anaerobic glycolysis, however. The most important point made by them is that in the tumors a large part, probably all, of the lactic acid is derived from glucose. In the normal adult liver this is not the case and, in fact, glycolysis is hardly increased at all by the presence of glucose (55) though it may be slightly increased in the fetal liver (54). Hence Orr and Stickland showed that a qualitative change of metabolism occurs when normal liver is transformed to tumor. Owing to scarcity of their material data on respiration are lacking, and those on aerobic glycolysis are solely of glucose breakdown. They showed, however, that aerobically as well as anaerobically glucolysis occurs in both cholangiomas and hepatomas.

In a preliminary account, which reached us when the present work was almost completed, Burk, Behrens, and Sugiura (4), indicate that they alone have made a complete study of the respiration, glycolysis, and R.Q. of *p*-dimethylaminoazobenzene liver tumors. In general, the anaerobic and aerobic glycolysis was intermediate in amount between those of the neoplasms investigated by Warburg and of the mouse tumors studied by Murphy and Hawkins and by Crabtree. "Compared to that of the original liver tissue, however, where the anaerobic lactic acid formation is almost negligible, the increase in the hepatic tumor is relatively very marked" (4). The R.Q. of the tumors was definitely less than unity.

We have extended the observations on liver and tumors derived therefrom in two directions: (a) In addition to measuring the respiration, glycolysis, and R.Q. of hepatic neoplasms induced by *p*-dimethylaminoazobenzene, we have studied for comparison the benign type of hepatoma occurring spontaneously in certain strains of mice. (b) Metabolic activities other than those mentioned above have been investigated in both groups of tumors.

It was thought that a comparison of the metabolism of the neoplasm with that of adjacent liver tissue should be extended to as many component reactions as possible, since the respiratory metabolism of a cell represents the sum total of the activities of a very large number of enzymes. Two tissues may have a superficially similar respiratory metabolism, yet there may be fundamental differences in the way the metabolic structure is built up, as gains on the one side may be compensated by losses on the other. Normal and cancerous liver tissues are particularly suitable for such a comparison owing to the many highly specialized functions performed by the normal liver.

Greenstein and his collaborators (25, 26, 27) have studied various enzymes in extracts of transplanted hepatic tumor and compared them with similar extracts of normal liver. In the tumor extracts, they found reductions in the activities of catalase, arginase,

and xanthine dehydrogenase. The activity of the enzyme thymonucleodepolymerase was only 10 per cent lower than that from normal liver, and no difference was found in the activity of "amylase." Kishi, Fujiwara, and Nakahara (34) prepared extracts from transplantable rat hepatoma and from normal liver and observed a definite lowering of cathepsin activity in the tumor extract, whereas dipeptidase and tripeptidase activities were equal in tumor and normal liver. Phosphatase from liver cancer induced by *p*-dimethylaminoazobenzene had an alkaline pH optimum, while the enzyme from normal liver had an acid pH optimum (42). Hepatoma and normal liver tissue also differ in their content of coenzymes, vitamins, etc. Thus the concentration of both ascorbic acid and glutathione is increased in a certain strain of transplantable rat hepatoma (22); this tumor is also rich in provitamin D, which is absent from *p*-dimethylaminoazobenzene hepatomas as well as from normal liver (35). No vitamin A could be detected in such hepatomas, or in four generations of transplants therefrom (23). Whereas the vitamin B_1 content of these hepatomas is normal, that of cocarboxylase is decreased (43). They contain about one-fifth as much cytochrome *c* (18) and about one-seventh as much lactoflavin (33) as normal liver. The induction of liver tumors in rats by the dye is marked by a decrease of arginase and histidase in the nontumorous liver tissue (41).

It will be seen that most of the catalysts hitherto studied are components of the enzymatic equipment common to all living cells. In addition to these, hepatic tissue contains some highly specialized systems that are either specifically localized in the liver or are shared, usually in a much less active form, by one or a few other tissues. Any diminution or loss of these highly differentiated functions would be a chemical indication of the degree of dedifferentiation [1] entailed in the process of neoplasia.

We have therefore investigated, by the tissue slice method, the following reactions; urea synthesis, deamination of amino acids, oxidation of fatty acids, oxidation of uric acid, and synthesis of glycogen. The tumor material was the same as that employed in the investigation of respiration and glycolysis; *i.e.*, rat hepatomas induced by feeding *p*-dimethylaminoazobenzene, and spontaneous hepatomas of old agouti mice.

SOURCES OF MATERIAL

Liver Tumors Produced by Feeding *p*-Dimethylaminoazobenzene

Feeding experiments.—Two groups of 30 black-hooded rats each were fed rice to which had been added 20 ml. per kg. of a warmed 3 per cent olive oil solution of dimethyl yellow (B.D.H. analar). Carrot was added at the rate of one small slice per rat per day. Water was given ad libitum and the food was always present in the cages. The rice used was Egyptian grown, of Japanese type, polished but not washed. Its vitamin B_1 content was estimated (53) and found to be 0.23 μgm. per gm.

The first group of 30 rats (Nos. 1 to 30) received the rice without pretreatment. The second group (Nos. 31 to 60) were fed rice which had been extracted with diethyl ether by refluxing the unground rice (1 kg. batches) with two lots of the solvent (1 liter each). The ether extract was filtered off and the rice dried thoroughly in the air before use. On evaporation the ether yielded a clear yellow oil amounting to 0.2 to 0.3 per cent of the weight of rice taken; the properties of this oil were not investigated in view of the absence of any striking effect of the ether extraction on the incidence, type, or metabolism of the tumors or on the metabolism of the livers. The addition of rice-bran oil (ether-soluble substance) to the basic rice diet has been found by Kinosita (33) and Sugiura and Rhoads (59) to have a definite inhibiting effect upon the production of liver cancer in rats by the oral administration of *p*-dimethylaminoazobenzene. It is evident, therefore, that the polished rice used did not contain sufficient, or the right kind of, oil which was readily extractable by ether for this preliminary extraction to affect the incidence of tumors. It is noteworthy that in both the investigations referred to above large quantities of rice-bran oil (1 cc. per rat per day) were given.

Tumor incidence.—50 per cent of the animals in each group died before the 100th day of feeding; most of these showed no liver changes of interest for the present purpose and were not further examined. This high mortality agrees with that reported by Harada, Mizuta, and Maruya (29) and by Nakahara and his associates (45), and differs from the good survival observed by Sugiura and Rhoads (59). There was an initial tendency to earlier death in the rats that received the ether-extracted rice. Cannibalism was not uncommon, and some material had to be discarded from this cause. Of a total of 22 rats which came to autopsy after having survived more than 140 days 19 had liver tumors, divided almost equally between the plain and ether-extracted rice groups (10 and 9 tumor-rats respectively). Of the ordinary rice group, 2 rats killed after 235 days had livers which were macroscopically normal as had also 1 rat of the ether-extracted rice group killed after 209 days. The other rats were killed at intervals for metabolic experiments between the 84th and 236th days.

Nature of the liver changes.—These have been so fully discussed (32, 40, 49, 21) that a brief reference

[1] This term is used throughout without implying any specific type of mechanism by which the undifferentiated cell may arise.

to the main points will suffice. With one exception the tumors were examined histologically, and in many instances the surrounding liver tissue also. For these examinations we are indebted to Professor A. F. Bernard Shaw and Dr. J. G. Thomson. Definite macroscopic changes were first apparent in animals that died or were killed from the 84th day onwards. Cirrhosis was absent from the greater proportion of our specimens; it appeared to have been much less common than the descriptions of Orr (49) indicate for his material. Only two livers, in rats killed early in the experiment (84th and 107th days), showed typical nodular cirrhosis. Three other rats at the same period showed bile duct hypertrophy, not yet neoplastic, but no cirrhosis. All tumors present after the 140th day were histologically malignant.

The question of the origin of the tumors is at present open to discussion. The differing views of Orr (49) and the Japanese workers have been reviewed and given a detailed investigation by Edwards and White (21), without any final decision having been reached. Thus we merely report the results of routine histological examination without necessarily implying any significance as to the tissue of origin. Current views may be briefly summarized by saying that while the hepatoma is generally conceded to be derived from the parenchyma, there is no such unanimity with regard to the bile duct origin of the tumors that have histologically the appearance of cholangiomas. The question is important since the object of this investigation was to compare the metabolism of the tumors with that of their tissue of origin, and the problem will be mentioned again after the experimental results have been described.

Here our material will be considered only on a conventional histological basis. From this point of view there was the following distribution:

Bile duct adenoma	2 rats
Bile duct adenoma with early carcinomatous change	6 rats
Bile duct carcinoma with transition to liver cell carcinoma	6 rats
Liver cell carcinoma	3 rats

Frequently the bile duct carcinoma in certain areas resembled liver cells, and in some instances apparent bile duct carcinoma was continuous with liver cell carcinoma. Sometimes, however, pure bile duct adenoma or carcinoma occurred, with no trace of liver cell carcinoma or transition thereto. In one such case special staining showed that many of the bile duct spaces and a number of the epithelial cells contained mucus, which may be an indication of their bile duct origin. We are indebted to Professor Shaw for this observation. The brief description applied to the material used for metabolic measurements, and included in the tables, is also based on these histological appearances.

Spontaneous Hepatomas of the Mouse

The tumors arose in old mice of Strong's CBA strain maintained by Mr. F. C. Pybus and Dr. E. W. Miller, to whom we are much indebted for this material. The occurrence and nature of these spontaneous hepatomas have been fully described by Strong and Smith (57, 58), Burns and Schenken (6), Gorer (24), and Pybus and Miller (51). The tumors, much more common in male than in female mice, were from 0.5 to 2.0 cm. in diameter, appearing usually as single masses projecting slightly above the surface of the organ and readily distinguished by their difference in color, and sometimes in texture, from the normal lobe. The surrounding liver was macroscopically normal. Microscopically the tumors are composed of liver cells (not bile duct), which differ but little in appearance from the normal. All the authors cited above agree on the generally benign tendency of the tumors. Thus Strong and Smith (57) state "The tumors seem to be of benign character, enlarging slowly by expansive growth." Later the same authors (58) successfully inoculated one into related mice; all the grafts were surrounded by well formed capsules. The liver tumors observed by Burns and Schenken (6) in C_3H mice "possessed some characteristics of both benign and malignant growths." A blood vessel was invaded in one instance, yet the "remarkable differentiation of the tumor cells, the infrequency of mitoses, the absence of metastases, and the failure or extremely slow growth of the tumor transplants were suggestive, on the other hand, of benign tendencies." Gorer (24) and Pybus and Miller (52) have observed pulmonary metastasis from one such tumor in each laboratory. In the latter metastasis, directly in the center of the lung, the architecture of the normal liver was preserved with astonishing completeness. We are specially indebted to Mr. Pybus and Miss Miller for directing our attention to this very interesting tumor, and to Dr. J. W. Orr for his comments; unfortunately there was no opportunity to undertake metabolic measurements with this specimen. The nature of these tumors may be summed up by saying that they are histologically almost identical with normal liver, but that they are capable of growth under favorable conditions when transplanted, and that very occasionally their metastasis has been observed. Their cause is unknown; leukocytic infiltration of the liver is sometimes present, and although a virus disease (infectious ectromelia) was present in some of Strong and Smith's (58) animals, the occurrence of virus and tumor could not be correlated.

METHODS FOR METABOLIC STUDIES

Preparation of the tissue.—The animals were killed by cervical dislocation when palpation, the lethargy of

the animals, or the duration of feeding in the *p*-dimethylaminoazobenzene group, suggested that liver tumors might be present. The livers were excised and used immediately, a specimen being retained for histological examination. Razor slices of tumor and liver were prepared in Ringer's solution as usual, the greatest care being taken to avoid the contamination of liver by adjacent tumor, and *vice versa*. Macroscopically it was easy, as a rule, to distinguish tumor from liver, but occasionally it was difficult, in the *p*-dimethylaminoazobenzene animals, to be certain that the organ was completely nontumorous, owing to the multiple nature of these growths. Sometimes they were too small or too diffuse for measurement, and these specimens were discarded. Sometimes only incomplete measurements could be made for a similar reason.

Respiration and glycolysis.—In the aerobic experiments 0.2 per cent of glucose was always present, but anaerobic glycolysis was frequently determined in both the presence and absence of glucose. For aerobic experiments in bicarbonate Ringer's solution (36) either the two-vessel method of Warburg (60) or, when the R.Q. was also to be measured, the method of Dickens and Šimer (12) was used. The gas phase was oxygen with 5 per cent CO_2. In accordance with convention the aerobic glycolysis ($Q_G^{O_2}$) in the Warburg two-vessel method is expressed on the assumption of an R.Q. of unity. This is obviously incorrect, but a simple calculation is all that is needed to transform the values of $Q_G^{O_2}$ for any chosen value of R.Q.

In the methods of Dickens and Šimer (10, 12) we have now used for a long time equal wet weights of tissue, thus dispensing with the need for a separate determination of the bicarbonate content of the solutions. Also, by using for the R.Q. in bicarbonate-Ringer solution the convenient type of vessel described by Dickens and Greville (9), the amount of Ringer's solution may be increased; normally we now use 2 cc. per vessel. Occasionally respiration and R.Q. in phosphate-Ringer solution (M/50 phosphate buffer, pH 7.4, in Krebs-Henseleit saline) were measured by the method of Dickens and Šimer (10) in pure oxygen.

Anaerobic glycolysis was determined manometrically (60), in an atmosphere of nitrogen with 5 per cent CO_2.

Glycogen was estimated by the method of Hynd and Rotter (31), with 1 gm. of fresh tissue, and by the Somogyi reagent (56) for estimating reducing power after hydrolysis.

Nucleic acid phosphorus was determined as described by Berenblum and his associates (1). The metabolic quotients (Q values) when expressed on the basis of nucleic acid phosphorus, in μgm., instead of dry weight of tissue, in mgm., are written PQ (1).

Special liver functions.—The tissue slices were shaken for 90 minutes with bicarbonate-Ringer solution to which the appropriate substrates had been added, in an atmosphere of oxygen containing 5 per cent CO_2, at 37.5° C. At the end of the incubation period the slices were removed, dried, and weighed, except in the experiments on carbohydrate synthesis. In these the results were based on the initial wet weight; in all other experiments they were based on the final dry weight.

After removal of the slices the solutions were analyzed by one of the procedures described below.

Urea synthesis.—As shown by Krebs and Henseleit (36), the synthesis of urea from added ammonium salts is optimal in the presence of traces of ornithine. The addition of a substrate, such as pyruvate or lactate, is favorable, too. Urea was determined by the method of Krebs and Henseleit (36), where the CO_2 liberated from urea by urease at pH 5 is measured manometrically. As our urease preparations (aqueous extracts of B.D.H. jack bean meal) showed a weak carboxylase activity, pyruvate somewhat interfered with the estimation of urea. *dl*-Lactate was therefore chosen as additional substrate; its final concentration was 1.74×10^{-2}M. Ammonium chloride was present in a concentration of 8.7×10^{-3}M and *l*(+)-ornithine dihydrochloride in a concentration of 8.5×10^{-4}M.

Amino acid deamination.—*l*(+)-Alanine was used as the substrate in a final concentration of 9.1×10^{-3}M. As the ammonia liberated from the amino acid is rapidly converted into urea by liver slices, it was estimated as urea.[2] *l*(+)-Ornithine dihydrochloride in a concentration of 8.9×10^{-4}M was added to insure the presence of sufficient catalyst for the maximal rate of urea synthesis. That under these conditions the rate of amino acid deamination is really the determining factor in normal liver is shown by the fact that the urea synthesis is much faster when ammonium ion is added than when alanine is added.

In some experiments the preformed urea was determined in both liver and tumor slices at the beginning of the experiment. Similarly, the formation of urea after 90 minutes' incubation without the addition of a urea precursor was measured. In both cases the amount of urea found was so small as to be almost within the limits of experimental error. The results obtained after 90 minutes' incubation with urea precursors therefore represent the true urea synthesis.

Fatty acid oxidation.—This was followed by measuring the formation of acetoacetic acid after incubation of the tissue slices with 4.4×10^{-3}M sodium caprylate. After removal of the slices at the end of the incubation period the solutions were acidified with 1/10 volume of 50 per cent citric acid and acetoacetic

[2] These results therefore measure oxidative urea synthesis from *l*-alanine, and only when the urea-synthesizing activity is retained are they also a true measure of deamination.

acid was measured manometrically at 25° by addition of the aniline citrate reagent of Edson (19).

Uric acid oxidation.—Lithium urate was added to a concentration of 2.5×10^{-3} M and its disappearance was measured colorimetrically. Trials with the manometric method of Edson and Krebs (20) were unsatisfactory. In this method, which is based on the conversion of uric acid into urea and glyoxylic acid, uric acid and allantoin have to be estimated separately. Uric acid is measured by the urea obtained after oxidation with MnO_2, followed by alkaline and finally by acid hydrolysis. Allantoin, on the other hand, is determined by the urea formed in an aliquot after alkaline and acid hydrolysis, but without previous treatment with MnO_2. We consistently found the allantoin too high, obviously because of an autoxidation of uric acid during the alkaline hydrolysis (20 minutes at 100°, pH about 10).

Uric acid was therefore estimated colorimetrically with the lithium arsenotungstate reagent of Newton (48). After removal of the slices, the solution (3.3 ml.) was deproteinized by the addition of 0.2 ml. of N H_2SO_4 and 0.3 ml. of 10 per cent sodium tungstate. The measured filtrate was made up to 25 ml. and uric acid determined in a 5 ml. aliquot. Five ml. of arsenotungstate reagent (diluted 1:5) were added together with 15 ml. of 5 per cent sodium cyanide in 25 per cent urea and water to make up to 50 ml. A uric acid standard of 0.2 mgm. was used for comparison.

Synthesis of fermentable carbohydrate.—The tissue slices used for this experiment were divided into two roughly equal portions, drained on filter paper, and rapidly weighed on the torsion balance. They were then transferred to two manometer vessels containing 3 ml. of bicarbonate-Ringer solution and 0.3 ml. of 0.2 M pyruvate. One lot, which served for the estimation of the preformed carbohydrate, was immediately acidified by the addition of 0.5 ml. of 10 N H_2SO_4. The other vessel was shaken for 90 minutes at 37.5° C. before acidification in the same way. After acidification the vessels were put into a boiling water bath for 10 minutes. The contents of each vessel were then transferred to a mortar, thoroughly ground, and finally poured into a large test tube; 5 ml. of water + 3 ml. of N H_2SO_4 were used, in several small portions, to rinse vessel and mortar. The test tubes were covered with glass bulbs and heated in a boiling water bath for 3 hours. After cooling, 1 ml. of 25 per cent NaOH and 1.3 ml. of 10 per cent sodium tungstate were added, the volume was measured, and the solution centrifuged. One ml. of acid cadmium sulfate solution [3] and 0.4 ml. of 25 per cent NaOH were added to the measured supernatant solution, it was thoroughly mixed, and again centrifuged. The clear solution was tested with a drop of dilute NaOH solution and addition of NaOH was continued, if necessary, until precipitation was complete. When this was the case, the filtrate was neutralized to litmus.

The estimation of reducing power was carried out in 5 ml. aliquots, before and after fermentation, with the reagent of Somogyi (56). For fermentation 5 ml. of a 10 per cent suspension of well washed baker's yeast were centrifuged and the solution to be fermented was added to the residue. After thorough mixing, the tube was left at room temperature for 15 minutes before it was again centrifuged and the residual reduction determined in the supernatant solution.

Units.—In order to conform with the notation of respiration and glycolysis all metabolic rates are expressed in Q values; *i.e.,* the amount of metabolite produced or consumed, in gaseous form at N.P.T., expressed in μliters (1 millimol = 22,400 μliters) per hour, per mgm. of dry weight of tissue. The amount of urea and acetoacetic acid is directly given by the quantity of CO_2 evolved, as one molecule of CO_2 is liberated from each molecule of these compounds. The rate of amino acid deamination is also expressed in terms of Q_{urea}, but it is worth bearing in mind that each molecule of urea indicates the deamination of two molecules of alanine.

The results of the carbohydrate analyses are expressed in terms of glucose. The initial and final concentrations of carbohydrate are given as percentages of the initial tissue wet weight, but the metabolic changes are again converted into Q values, the final dry weight of tissue being here taken to equal 0.2 initial wet weight.

METABOLISM OF TUMORS INDUCED BY *p*-DIMETHYLAMINOAZOBENZENE

Ten histologically malignant tumors were obtained of size and purity sufficient for investigation. In three of these (Nos. 52, 53, 54) a full examination was possible, including respiration, glycolysis, and R.Q. of tumor and (except No. 53) liver, as well as urea formation, deamination, oxidation of fatty acid and uric acid, and synthesis of carbohydrate, in both tumor and liver. In the other examples the amount of available tissue limited the extent of the investigation to selected metabolic activities. Respiration and glycolysis of livers from rats 25, 26, 54, and 57 were compared with those of the tumors arising in these organs; stages preceding the formation of malignant tumors were studied in the livers of rats 15, 16, 24, 28, and 30; and respiration and glycolysis of the macroscopically tumor-free parts of the livers of rats 16, 25, 26, 54, and 57 were also measured. Metabolic activities other than respira-

[3] Acid cadmium sulfate solution: 13.0 gm. cadmium sulfate and 63.5 ml. N H_2SO_4 in 100 ml.

tion and glycolysis were studied in both liver and tumor of rats 21, 52, 53, 54, 56, and 59.

The results of measurements of respiration and glycolysis are collected in Table II, while those of the other metabolic functions are given in Table III.

TABLE II: RESPIRATION, R.Q., AND GLYCOLYSIS OF LIVER TISSUE AND LIVER TUMORS OF RATS FED *p*-DIMETHYLAMINOAZOBENZENE

Rat No.*	Method	Time of experiment, hours	$-Q_{O_2}$	$Q_G^{O_2}$	$Q_G^{N_2}$	R.Q.	$Q_G^{N_2}$ (no glucose)	Glycogen, per cent	Nucleoprotein-P,† per cent	Remarks
A. MACROSCOPICALLY NONTUMOR PART OF LIVERS										
16	W	⅓	11.2	−0.8	+ 5.8	—	—	—	0.38	No cirrhosis
25	W	1½	9.4	+1.0	+ 5.3	—	+5.9	0.28	0.35	No cirrhosis or erythroblastic foci
26	W	1½	8.4	+0.6	+ 6.3	—	+5.2	0.45	0.37	No cirrhosis, slight bile duct proliferation
57	D & S	2	11.0	+0.94	+ 3.5	0.92	+3.7	—	0.18	" " " " " "
54	D & S	2	11.0	+0.65	+ 5.2	0.86	+3.7	—	0.28	No cirrhosis
Average			10.2	+0.5	+ 5.2	0.89	+4.6	—	0.31	
B. VARIOUS INTERMEDIATE STATES										
15	W	1	8.0	−3.2	+ 3.0		—	—	0.34	Cirrhosis
			15.4	−1.1	+ 3.3					
24	D & S	3	9.8	+2.0	+ 5.4	0.84	+3.8	—	0.29	Areas of bile duct proliferation
			9.5	+2.2	+ 6.8	0.81	+4.0	—	0.32	
			7.2	+2.7	—	0.78	—	—	—	
30	D & S	2	10.7	+3.0	+ 6.2	0.87	+3.3	—	0.37	Diffuse bile duct proliferation
			10.5	+3.4	+ 8.6	0.86	+3.7	—		
28	W	2	10.5	+4.6	+ 6.2	—	+4.4	1.02	0.35	" " " "
16	W	⅓	13.2	+2.6	+16.0	—	—	—	0.55	Nonneoplastic bile duct adenomatous growth
Average			10.5	+1.0	+ 6.2	0.83	+3.8	—	0.34	
C. LIVER TUMORS										
28	W	2	—	—	+ 9.6	—	+3.7	—	0.42	Bile duct adenoma and (?) early carcinoma
25	W	1½	6.1	+5.9	+17.4	—	+1.8	0.11	0.46	Bile duct adenoma and carcinoma
26	W	1½	6.8	+6.1	+14.1	—	+1.9	0.16	0.46	" " " " "
53	D & S	2½	9.9	+4.9	+10.0	0.86	+2.1	—	0.58	Pure bile duct adenoma to carcinoma (mucus-containing)
			8.2	+5.3	—	0.89				
57	D & S	2	15.0	+7.8	+14.2	0.86	+1.5	—	0.51	Bile duct adenoma and liver cell carcinoma
54	D & S	2	12.2	+6.6	+ 8.7	0.90	+4.0	—	0.38	" " " " " " "
52	D & S	2	17.8	+5.9	+22.3	0.60	+2.1	—	0.65	Liver cell carcinoma
			20.3	+6.2	—	0.61				
Average			12.0	+6.1	+12.5	0.79	+2.5	—	0.49	

W = Two-vessel method of Warburg (60); D & S = bicarbonate R.Q. method of Dickens and Šimer (12).

* Rats 1 to 30 received ordinary rice; rats 31 to 60 had ether-extracted rice.

† Since the nucleic acid metabolic quotients (PQ values) are based on the μgm. nucleic acid phosphorus, while the ordinary metabolic quotients (Q values) are based on the mgm. dry weight of tissue, the conversion of the above Q values to PQ values is given by:

$$PQ = Q/10p,$$ where p is the percentage of nucleic acid phosphorus

based on the dry weight of tissue (penultimate column).

RESPIRATION AND GLYCOLYSIS

None of the livers examined can be regarded as strictly normal, in view of the prolonged administration of dye that preceded all experiments. Metabolically, however, there is little or no change in any of the "nontumor portions of liver" included in group A of Table II from the values typical of liver from normal rats on an adequate diet (13). The respiration and low aerobic acid production are certainly close to their expected magnitudes. The anaerobic glycolysis in normal liver is a somewhat uncertain quantity, owing to its dependence on the glycogen content (50) and previous treatment of the slices (55). The anaerobic glycolysis of adult liver is, however, predominantly a glycogenolysis (55), being little affected by the presence or absence of glucose in the medium. This is also true of the nontumor *p*-dimethylaminoazobenzene livers of Table II with the possible exception of No. 54, which apparently had some slight glucolytic as well as glycogenolytic power.

The states described as "intermediate" in Table II, group B, represent a collection based upon histological examination, which revealed at most a condition that might be described as an adenomatous growth of bile ducts without any histological indication of malig-

nancy. One example of cirrhosis which, as already mentioned, was rare in our series, has also been included. The duplicate estimations quoted for this specimen (No. 15) appear to show a considerable difference in metabolic activity in various regions of this liver; otherwise it was metabolically not unlike the normal organ. The other examples showed varying degrees of bile duct proliferation, and their metabolism (Nos. 24, 30, 28, and 16) shows no special change of respiration and R.Q. when compared with the normal. In all these examples, however, there is evidence of an appreciably increased aerobic acid formation, and in one instance (No. 16) of a greatly augmented anaerobic glycolysis compared with the nontumor portion of the same liver (Table II). In the remaining examples of this series the magnitude of $Q_G^{N_2}$ is not appreciably different from that of the nontumor parts, but it is probably significant that in each of the three cases studied (Nos. 24, 30, and 28) the anaerobic glycolysis is higher in the presence of glucose than in its absence. It seems that even in this early phase the cells of the liver have commenced to turn towards glucose in their anaerobic metabolism. This and the increased aerobic glycolysis are the only significant alterations at this stage.

The group (Table II, C) headed "liver tumors" includes only those examples which showed histological evidence of malignancy. On this basis they fall into two broad subgroups: (a) histologically bile duct carcinomas (Nos. 28, 25, 26, 53); (b) bile duct carcinomas containing also liver cell carcinoma (Nos. 57, 54, 52). The controversial nature of the interpretation of the histological picture in terms of the origins of the cells of these tumors has already been pointed out.

All the tumors of this group were alike in the following respects. The aerobic glycolysis was in excess of that found for tissues of groups A and B; this excess was in every case quite clearly marked when compared with group A, and although not large for tumor 53 (with lowest $Q_G^{O_2}$ of group C) compared with intermediate state 28 (with highest $Q_G^{O_2}$ of group B), the general tendency to higher aerobic glycolysis in the malignant tumors was quite uniform. The increase in anaerobic glycolysis was usually well marked in the malignant tumors, and this difference becomes more striking when their relatively low glycolysis in the absence of glucose is taken into account. Tumors 25, 26, 53, 57, and 52 have turned almost exclusively to glucolysis as their anaerobic energy source. Curiously, tumor 54 (*cf.* incomplete measurements on tumor 28), which was an advanced tumor of considerable size and histologically undoubtedly malignant, had an anaerobic metabolism almost identical with that of the early bile duct proliferation of intermediate state 30; comparison with the nontumor hepatic tissue of the same animal (No. 54, A and C) showed, however, some increase of anaerobic glycolysis and a 10-fold increase of aerobic glycolysis in the tumor. These determinations were run side by side and for the same duration; they are thus strictly comparable. The R.Q. of the liver tumors is of an order and range similar to that of normal liver, and resembles that of other tumors (11, 13, 5) in being below unity.

A comparison of the neoplasms according to their predominantly bile duct or liver structure shows that the only important difference in the two subgroups is a tendency to a lower respiration in the former (Nos. 28, 25, 26, 53) compared with the latter (Nos. 57, 54, 52). Tumors 57 and 52 had a particularly high Q_{O_2}, even though this was the average over 2 hours during which the respiration certainly fell somewhat; and the difference is not entirely explained by the increased cellularity of these tumors as indicated by their high content of nucleoprotein-phosphorus (*cf.* Table I, penultimate column). The histologically pure bile duct carcinoma of rat 53 had nucleoprotein-P intermediate in amount between liver cell tumors 57 and 52, but nevertheless had only about half their respiration. As far as the present data go, they indicate a high respiration of carcinoma cells of liver cell type. On the other hand, comparison of liver and tumor from Nos. 25 and 26 shows that the bile duct type of tumor has a lower respiration than that of the nontumorous liver tissue of the same animal, in spite of the higher nucleoprotein-P content of the tumor tissue.

Special liver functions.—Six tumors could be investigated (Table III). In 4 the whole series of functions could be studied, but in two the tissue available was sufficient only for the measurement of urea synthesis, deamination, and fatty acid oxidation. In three instances (Nos. 52, 53, 54) respiration, glycolysis, and R.Q. were determined on the same material. The tumors represented all histological types, ranging from structures resembling bile duct adenoma and carcinoma to pure liver cell carcinoma. There was, however, no clear cut difference in the metabolism of these histologically different types.

All the metabolic functions studied here were greatly reduced or had been lost entirely in the tumor tissue. But it is of interest that these functions disappear by no means in the same degree. The urea synthesis in particular usually persists at a measurable, though greatly reduced, rate, even after other reactions have ceased. This seems to favor a parenchymal cell origin for these tumors. They appear to be at different levels of dedifferentiation; thus tumor 52, which also has a relatively well conserved power of urea synthesis, still shows a considerable activity of deamination and fatty acid oxidation, though these are absent from some other tumors. No. 56 displays high

uricase activity, yet is unable to deaminate alanine or oxidize caprylic acid. In other cases there is not only no disappearance of uric acid but even an increase, as indicated by the positive quotient.

There is some variation, too, in the figures for the nontumorous livers. But though this was not neoplastic, it was not normal either, showing various degrees of degenerative, hypertrophic, or cirrhotic change. It must be remembered, furthermore, that certain activities, such as acetoacetic acid formation and glycogen synthesis, depend to some degree on the nutritional state of the liver. This is especially true for glycogen synthesis, which is most active if the liver contains little preformed carbohydrate (rat 59). The carbohydrate content of the tumors, though at a consistently very low level, is less variable than that of the surrounding liver, suggesting that it is less readily mobilized. Whereas all livers showed a considerable increase of fermentable carbohydrate after incubation with pyruvate, there was a decrease in two tumors. Two others exhibited a very small increase, almost within the limits of experimental error. Even if these increases be real, they show at least a greatly reduced capacity of synthesizing carbohydrate.

The objection may be raised that the small residue of the special hepatic functions which the tumor slices still possess is in reality due to contamination with surrounding liver tissue. This is very unlikely, as the tumor slices were prepared only from the central parts, all marginal portions being rejected. No islands of normal liver tissue were observed histologically inside the tumor. Furthermore, assuming an even distribution of this error, all values should be reduced to a similar degree, which is not the case.

Table III: Analysis of Some Special Aspects of Liver Metabolism in *p*-Dimethylaminoazobenzene Tumors and in Adjacent Nontumorous Liver of the Rat

Animal No.	Tissue	Urea formation from NH_4^+ Q_{urea}	Urea formation from alanine Q_{urea}	Acetoacetic acid formation from caprylate $Q_{acetoac}$	Uric acid oxidation $Q_{uric\ acid}$	Total fermentable carbohydrate: Initial, per cent	Total fermentable carbohydrate: After 90 minutes, per cent	Synthesis of carbohydrate Q_{CH}	Microscopic diagnosis of tumor
21	Liver tumor	4.3	0.9	4.5					—
		1.5	0	0					
52	" "	9.3	3.2	5.9					Liver cell carcinoma
		2.0	0.9	1.2					
53	" "	12.6	2.4	3.1	−1.7	1.78	2.16	+1.5	Bile duct adenoma to carcinoma
		0.3	0	0.5	+1.4	0.14	0.042	−0.40	
54	" "	9.6	1.9	4.3	−4.0	0.39	0.51	+0.51	Bile duct adenoma to carcinoma
		0.6	0.3	0.3	± 0	0.13	0.034	−0.40	+ liver cell carcinoma
56	" "	11.9	2.8	6.6	−4.0	1.61	2.03	+1.8	" "
		0.7	0	0	−3.4	0.027	0.055	+0.10	
59	" "	9.0	2.7	7.7	−1.9	0.092	0.94	+3.5	Bile duct carcinoma in places approaching liver cells in structure
		0.8	0	0.5	+2.5	0.23	0.29	+0.25	
Mean	Liver tumor	9.5	2.3	5.3	−2.9	0.97	1.41	+1.8	
		1.0	0.02	0.42	+0.12	0.13	0.105	−0.11	

METABOLISM OF SPONTANEOUS HEPATOMAS OF MICE

The material was never sufficient for the measurement of respiration and glycolysis, and of the other metabolic activities studied, to be carried out on one and the same tumor. The similarities in the results obtained with different tumors in each series, however, make it extremely probable that these neoplasms have in general a fairly constant type of metabolism, and therefore the results of the two sets of investigations—of respiration and glycolysis, and of the other metabolic activities—may reasonably be compared one with another.

Respiration and Glycolysis

The results for 7 tumors and the normal livers of their hosts are collected in Table IV. The most striking feature is the general similarity in the metabolism of liver and tumor, and this result contrasts strongly with the undoubted change of metabolism found in the highly malignant tumors of the rat (Table II) induced by *p*-dimethylaminoazobenze.

Considering the results in detail, there are pronounced individual differences but none of these is systematically present, and it is highly probable that they are adventitious and to a large extent due to unavoidable chance selection of necrotic areas and other similar factors. It is known that in these livers and tumors pyknosis and other degenerative changes are frequent. This probably accounts for the fact that in some tumors the respiration is lower than that of the liver, and *vice versa,* and similar variations occur also in aerobic and anaerobic glycolysis.

In all examples except No. 4, the aerobic glycolysis

of these tumors (and livers) is negligible. It is perhaps of importance that tumor 4, which showed considerable aerobic glycolysis in contrast to the liver tissue of the same animal, was permeated by polymorphonuclear leukocytes, especially lining the sinusoids, and showed greater variation than usual in the size and shape of the nuclei, of which many were hyperchromatic. Since that these neoplasms still perform all the functions of normal liver, as far as they were studied, at a rate of normal or only slightly impaired activity. Perhaps the very low level of carbohydrate in the normal liver of animal 12, compared with that of the hepatoma, again indicates that the carbohydrate of the hepatoma is less readily mobilized in response to peripheral demands.

TABLE IV: RESPIRATION, GLYCOLYSIS, AND R.Q. OF SPONTANEOUS HEPATOMAS OF MICE, AND OF LIVERS OF THE SAME ANIMALS

			Hepatoma					Normal lobe				
Mouse No.	Method	Time of experiment, minutes	$-Q_{O_2}$	$Q_G^{O_2}$	$Q_G^{N_2}$	R.Q.	Nucleoprotein-P, per cent	$-Q_{O_2}$	$Q_G^{O_2}$	$Q_G^{N_2}$	R.Q.	Nucleoprotein-P, per cent
1	W	0–40	8.0	−0.1	+2.1	—	—	7.5	+0.1	+4.2	—	—
2	W	0–30	16.9	+1.1	+7.9	—	—	14.3	+1.2	+9.6	—	—
		30–60	16.4	−0.2	+3.4			15.4	−0.3	+3.8		
3	D & S	0–120	8.9	—	—	0.83	0.53	9.3	—	—	0.90	0.52
		0–120	8.7	—	—	0.81		8.7	—	—	0.88	
4	W	0–30	7.9	+5.0	+6.2	—	0.37	12.9	−2.2	+3.1	—	0.35
		30–60	9.3	+5.6	+6.0			12.4	−1.5	+3.2	—	
5	W	0–60	13.4	−3.3	+1.7	—	—	12.0	−1.2	+2.2	—	—
6	D & S	0–120	5.5	—	—	0.72	0.54	9.9	—	—	0.67	0.49
	W	0–60	6.1	−2.4	+0.9			13.0	−6.3	+1.6		
7	W	0–60	13.6	0.0	+3.7	—	0.30	19.4	−0.2	+1.4	—	0.28

W = Two-vessel method of Warburg (60); D & S = phosphate-R.Q. method of Dickens and Šimer (10).

TABLE V: ANALYSIS OF SOME SPECIAL ASPECTS OF LIVER METABOLISM IN SPONTANEOUS MOUSE HEPATOMAS, COMPARED WITH ADJACENT NONTUMOROUS LIVER

Mouse No.	Tissue	Urea formation from NH_4 Q_{urea}	Urea formation from alanine Q_{urea}	Acetoacetic acid formation from caprylate $Q_{acetoac}$	Uric acid oxidation $Q_{uric\ acid}$	Total fermentable carbohydrate: Initial, per cent	Total fermentable carbohydrate: After 90 minutes, per cent	Synthesis of carbohydrate Q_{CH}
8	Liver tumor	12.0						
		12.8						
9	" "			6.5				
				3.6				
10	" "	7.3	3.8	4.3	−3.6			
		5.8	2.6	3.9	−2.4			
11	" "	9.8	5.0	3.4	−7.1			
		7.9	3.0	3.8	−7.0			
12	" "					0.07	0.66	+2.4
						0.41	0.73	+1.3

leukocytes have been reported (39) to possess high aerobic glycolysis in Ringer's solution, it is possible that part of the metabolic activity of this specimen may be accounted for by the infiltration.

In general, however, the respiration, glycolysis, and R.Q. of these tumors are of the same type as those of normal mouse liver.

Special liver functions.—Unfortunately the number of tumors available for the investigation of special liver functions was small, and these usually did not yield enough slices for the whole series of analysis. But such experiments as there are (Table V) quite clearly show

METABOLISM OF NORMAL SKIN, LEUKOPLAKIA, AND CARCINOMA, OF THE VULVA

This human material was selected because the whole series of changes from normal, through the intermediate leukoplakia which is widely regarded as precancerous, and finally to squamous cell carcinoma may be followed. We are indebted to the Gynaecological Department of the Royal Victoria Infirmary, and especially to Dr. S. Way, for this material, which was used fresh immediately after excision in the form of razor slices. The subcutaneous fatty layer was carefully separated from the cutis and the latter was cut into

pieces of 50 to 200 mgm. dry weight. These large weights were necessitated by the low metabolic activity of skin and it is not considered that the R.Q. values, which averaged 1.0, are sufficiently reliable for reproduction here. The error is mainly in the variation of preformed CO_2, which was large compared with the respiratory CO_2. Similar errors occurred in attempts the excised material did not provide sufficient normal skin for this comparison. Normal skin of the vulva has a low metabolism, whether this is expressed as Q or as PQ values (Table VI). If the activity on a dry weight basis were about 10 times greater, the Q values would be nearly the same as for rat brain; *i.e.*, the relative values of respiration and aerobic and anaerobic

TABLE VI: METABOLISM OF NORMAL SKIN, LEUKOPLAKIA, AND CARCINOMA, OF THE VULVA

Case No.	Method	Time of experiment, minutes	$-Q_{O_2}$	$Q_G^{O_2}$	$Q_G^{N_2}$	R.Q.	$-PQ_{O_2}$	$PQ_G^{O_2}$	$PQ_G^{N_2}$
				NORMAL SKIN OF VULVA					
3	W	0–120	0.74	+0.20	+0.89	—	0.62	+0.17	+0.71
4	D & S (a)	0–120	0.64	1st hour	+1.05				+0.87
				2nd hour	+0.79	—	0.53	—	+0.66
6	D & S (b)	0–120	1.08	—	—	—	1.08	—	—
			0.93	—	—	—	0.93	—	—
7	W	0–60	0.92	+0.33	+0.72	—	1.5	+0.54	+1.2
Mean			0.86	0.27	+1.15		0.93	+0.36	+0.89
				LEUKOPLAKIA OF VULVA					
1	W	0–60	2.14	+2.60	+8.3	—	—	—	—
			1.78	+2.39	+7.1	—	—	—	—
5	W	0–90	1.27	+0.31	+1.15	—	0.85	+0.21	+0.77
	D & S (a)	0–105	0.69	—	—		0.46	—	—
			0.87	—	—		0.58	—	—
			0.93	—	—		0.62	—	—
8	W	0–60	1.36	+1.29	+3.43	—	1.07	+1.02	+1.91
Mean			1.29	+1.65	+5.00	—	0.72	+0.62	+1.34
				SQUAMOUS CARCINOMA OF VULVA					
2	W	0–60	2.23	+13.3	+21.6	—	—	—	—
			1.79	+13.8	+24.8	—	—	—	—
3	D & S (b)	0–135	2.55	+11.0			0.95	0.94	+4.06
			2.44	+10.4	+23.2		0.95	0.90	+3.84
			2.39	+13.6			0.88	0.88	+5.02
									+8.58
4	D & S (a)	0–120	3.52	— 1st hour +14.8		0.95	1.30	—	+5.48
			3.90	— 2nd hour +11.4		0.88	1.44	—	+4.22
Mean			2.69	+12.4	+20.7	0.92	1.09	+4.31	+6.09

W = Two-vessel method of Warburg (60); D & S (a) = phosphate-R.Q. method of Dickens and Šimer (10); D & S (b) = bicarbonate-R.Q. method of Dickens and Šimer (12). The PQ values are the Q values calculated on the basis of μgm. of nucleic acid phosphorus, instead of on a dry weight basis (*cf.* Berenblum, Chain, and Heatley, 1). Nucleic acid-P in the carcinoma of case 3 was assumed to be the same as carcinoma of case 4; all other nucleic acid-P values were determined.

to measure the R.Q. of leukoplakia (*e.g.*, triplicates, 0.94, 0.98, 1.06), and these measurements have also been omitted from Table VI, which summarizes the results. The experimental conditions were the same as those previously described, and the Ringer's solution contained 0.2 per cent glucose.

Four samples of skin, 3 of leukoplakia, and 3 of carcinoma were examined. In two cases skin and carcinoma from the same patient were used; in the others glycolysis are about the same in these two normal tissues. The PQ values may be compared with those of Berenblum, Chain, and Heatley (2) for normal isolated epithelium of the rabbit's ear, *viz.* $PQ_{O_2}-0.9$; $PQ_G^{O_2}+0.45$; $PQ_G^{N_2}+1.3$; (R.Q. 0.7). In view of the different material the correspondence is so good that here at least there seems little need of separating the nearly pure epithelial layer and using the elaborate ultra-micromethods of Berenblum and his colleagues,

since the much more readily applicable Warburg technic gives substantially the same results in this case.

The Q values of leukoplakia all tend to be increased as compared with skin, particularly the anaerobic, and to a less extent the aerobic, glycolysis. The PQ values of aerobic and anaerobic glycolysis are also increased. However, these changes are slight compared with the entirely different type of metabolism shown by the carcinoma. Here the Q values of respiration are only about double those of the leukoplakia, but the anaerobic and aerobic glycolyses have both risen to the high values typical of the majority of malignant tumors.

Naturally, since the *relative* magnitudes of the Q values are quite different in the tumors, compared with their relative values in skin and leukoplakia, the PQ values show the same kind of alteration. Considering the *absolute* increases, when these are based on the nucleic acid phosphorus as a supposed indication of the metabolically active tissue substance only, it is seen that the increase in aerobic glycolysis (0.4 to 4.3) and anaerobic glycolysis (0.9 to 6.1) is very great in the carcinoma compared with normal skin. Thus the metabolism of the tumor is not only qualitatively different in type from that of the skin, but the activity of its cellular elements appears to be much greater than that of the cellular material in skin.

These findings are in complete contrast to those of Berenblum and his associates (2) with Shope papilloma and rabbit epithelium. These authors' values for the papilloma are in good general agreement with those obtained by us for human leukoplakia, and the question arises whether the tumors used by them were really malignant. In view of our results, it is very desirable that this point should be investigated with histologically controlled material, and the changes followed right through to the stage of malignancy.

DISCUSSION

Histologically the two types of liver tumors studied in this investigation stand at opposite poles. Whereas the highly differentiated spontaneous mouse hepatomas closely imitate the structure of normal liver, the growths induced by *p*-dimethylaminoazobenzene are, at least in their final stages, definitely malignant and are composed of cells of a much more primitive anaplastic type. Whether these cells are originally derived from bile duct epithelium or from parenchymal cells is not of great importance from our point of view, since they might be expected to revert ultimately to the same primitive type, in view of their common embryological origin.

Our studies reveal the same picture in the field of metabolism; the spontaneous mouse hepatomas are almost indistinguishable from normal liver, not only with respect to respiration and glycolysis, but also in their capacity to perform some very specialized and organ-specific functions. The *p*-dimethylaminoazobenzene tumors, on the other hand, differ radically from normal liver in the following ways: They have acquired the power to ferment glucose both aerobically and anaerobically, a property common to almost all malignant growths. Though the extent of their respiration is not much changed, they retain only a small residue of, or have completely lost, the specialized oxidative functions particular to liver cells; *viz.*, formation of urea from *l*-amino acids, oxidation of fatty acids and of uric acid, and also other synthetic functions which depend on respiration: synthesis of carbohydrate and urea. This result shows how superficial an impression a mere recording of the net oxygen uptake gives of the respiratory activity of any tissue. Nor does the respiratory quotient in these cases where the R.Q. of the original tissue is low give any indication of the deep seated metabolic changes which have occurred.

The *p*-dimethylaminoazobenzene tumors thus present a metabolism which is highly dedifferentiated compared with that of normal liver. The appearance of fermentative glucose breakdown which persists in oxygen is, in our view, merely another aspect of the same transformation, or reversion to a more primitive type of metabolism, such as is shown by fibroblasts and epithelial cells when these are grown in tissue cultures (61, 37, 38).

Two objections may be raised against this view: (a) Aerobic glycolysis is lacking in the whole embryo, except in very early stages and then only after removal from the embryonic membranes (47), and it is also absent from embryonic liver or kidney (55) although embryonic liver still retains some ability to attack glucose anaerobically (54). (b) Normal adult tissues exist which have a high aerobic glycolysis, although they are highly differentiated (see Introduction).

These objections, however, are not necessarily incompatible with the general conception advanced, since (a) even the very young embryo already possesses some degree of differentiation; (b) the aerobic glycolysis of certain normal adult tissues, of which we have made a special study (14, 15, 16), may perhaps be regarded as an adaptation to the inadequately oxygenated environment in which these cells are situated within the body, as we have previously suggested. Thus the reversion to fermentation as a primitive source of energy in these normal tissues is not necessarily bound up with an all-round dedifferentiation. In the malignant tumor cell, on the other hand, the primary change is associated with an all-round dedifferentiation including, amongst other things, a reversion to the primitive type of fermentative metabolism.[4]

[4] It should be noted that some of the "histologically typically malignant" mouse tumors of Murphy and Hawkins (44) had no aerobic glycolysis, so it cannot be asserted that aerobic glycolysis is always a necessary consequence of this reversion. Direct evidence of the state of tissue oxygenation *in vivo*, in relation to tissue metabolism, is urgently needed in all these cases.

The extent of this reversion of metabolism in tumor cells varies, in the examples studied in this paper, in proportion to their deviation in morphological character and in invasive growth from the properties of normal tissue; that is to say, it varies by and large with the extent to which these cells have taken on the character of malignancy. Considered from this point of view, the nonglycolyzing spontaneous mouse hepatomas and leukoplakias of the vulva are examples of conditions which still retain a high degree of differentiation; their growth is orderly and the blood supply may therefore be adequate. In these cases it is only when the normal differentiation of the cell is lost, *i.e.*, at a stage which corresponds in general with the onset of malignancy, that the metabolism is definitely changed, and we consider it probable that malignant transformation *always* implies a change of metabolism, even if the over-all values of respiration, glycolysis, and respiratory quotient should remain unaltered. In such cases only a detailed study of the enzyme systems which make up the metabolic equipment of the cell will reveal the changes that have occurred.

In previous discussions much controversy has centered around the question of a specific tumor metabolism. There is no known single feature of tumor metabolism that is not possessed by one or other type of normal cells; on the contrary, the tumor cell has *lost* most of the specialized functions of normal cells: hence there is little reason to expect any one metabolic property to be exclusively characteristic of the tumor cell. In fact, no such specific property has been found.

It is to be expected that any biological difference between any two cells will ultimately be reflected in their chemistry and metabolism. The closer the biological relationship, however, the more difficult will be the detection of these subtle chemical differences.

SUMMARY

The metabolism of tumors of the liver and skin has been studied in relation to that of their tissue of origin.

Two types of liver tumor have been used: (a) malignant liver tumors induced by feeding *p*-dimethylaminoazobenzene to rats; (b) highly differentiated spontaneous hepatomas arising in agouti mice.

The skin tumors studied were carcinomas of the human vulva, and these were compared with the intermediate leukoplakia and normal skin of the vulva.

The respiration, glycolysis, and respiratory quotient of malignant liver tumors were similar to those found generally in tumor metabolism; *viz.*, high anaerobic and moderately high aerobic glycolysis combined with an R.Q. below unity. In confirmation of Orr and Stickland (50), it was found that in these tumors the glycogenolysis typical of normal adult liver is replaced by glucose breakdown. Precancerous states of the liver induced by *p*-dimethylaminoazobenzene, showed only a slight increase of aerobic glycolysis, and in some cases a slight anaerobic glucolysis also. The benign spontaneous mouse tumors had respiration, glycolysis, and R.Q. substantially the same as that of the adjacent liver tissue.

In addition to the measurements just described, the following highly specialized functions were studied in both types of hepatic tumor, and were compared with the surrounding nontumorous liver tissue: formation of urea from ammonia, formation of urea from *l*(+)-alanine, formation of acetoacetic acid from caprylic acid, oxidation of uric acid, synthesis of fermentable carbohydrate from pyruvic acid.

These highly differentiated functions were entirely, or almost entirely, lost in the malignant tumors induced by *p*-dimethylaminoazobenzene, though urea synthesis usually persisted at a measurable but greatly reduced rate even after the other reactions were lacking. On the other hand, the spontaneous mouse hepatomas still retained almost intact all the foregoing functions of the normal liver cell.

The respiration and glycolysis of carcinoma of the vulva were typical of those of malignant growths in general, both aerobic and anaerobic glycolysis being high. On the other hand, the leukoplakia showed only a slight increase of both the latter reactions as compared with the normal skin of the vulva.

The interpretation of none of these results is affected by the use of the nucleic acid phosphorus content of the tissue, instead of its dry weight, as a basis for the calculation of the metabolic values.

The loss of special liver functions by the *p*-dimethylaminoazobenzene tumors is regarded as a reversion to a more primitive type of metabolism. It is suggested that the appearance of glucolysis, aerobically and anaerobically, in this as well as in other tumors, is a metabolic expression of the same primary process of dedifferentiation.

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Studies in Esterase (Butyric) Activity

II. Esterase Content of Livers of Mice and Its Excretion in Strains Susceptible or Insusceptible to Mammary Cancer*

R. G. Chitre, Ph.D., and V. R. Khanolkar, M.D.

(From the Tata Memorial Hospital for the Treatment of Cancer and Allied Diseases, Bombay, India)

(Received for publication August 31, 1942)

In a previous publication (5) the esterase activity in the serum of a strain of highly inbred laboratory mice that are insusceptible to mammary cancer was shown to be almost double the amount normally found in two susceptible strains. This difference is a resultant of the following three interrelated factors probably working independently of each other: (a) the amount of enzyme elaborated in the liver, (b) its liberation in the circulating blood, and (c) its elimination from the body. It was suggested either that the mice of the insusceptible strain did not produce this enzyme in the liver to the same extent as the susceptible mice or that, even though the mice of these three strains manufactured the enzyme to the same extent, they released it in different proportions in the blood. The following experiments were therefore undertaken to determine the esterase content of the livers and the esterase excretion in the urine and the feces of mice from these three strains.

EXPERIMENTAL

Esterase Content of Liver

Material.—Ten virgin females and 11 males from each of the three highly inbred strains, C57, C3H, and A, described in our previous paper (5), were used. These were between 6 and 8 months old.

Determination of esterase.—The method used was that of Cajori and Vars (2) with a slight modification. The animals were killed by a blow on the head and the liver was removed as quickly as possible. The gall bladder was separated and the liver tissue was wiped with filter paper to remove the blood adhering to the surface. The liver was then dried in acetone for 24 hours. The next day it was taken from the fluid and the traces of acetone were completely removed in a vacuum desiccator. The dry tissue was weighed, powdered, and extracted for 24 hours at 37.5° C. with 10 cc. of 0.025 M ammonia. A drop of toluene was added as a preservative. The extract was acidified to methyl red indicator and centrifuged. Two cc. of the supernatant clear fluid were mixed with 10 cc. of phosphate buffer (pH 8.9) and 0.2 cc. of ethyl butyrate. The mixture was incubated for 1 hour at 37.5° C. The liberated butyric acid was titrated against decinormal sodium hydroxide. In every determination the necessary blanks were run as controls. The results are expressed in terms of cubic centimeters of decinormal NaOH required for one gram of the dry tissue.

In Table I the results of the esterase activity of the livers of these strains are shown, together with their means and standard errors. The statistical treatment was carried out according to the method suggested by Hill (4). From Table I it will be seen that the livers of the three strains studied are equally potent as regards this enzyme activity.

Excretion of Esterase

These findings led to a study of the excretion of this enzyme in the three strains of mice. It was thought preferable to carry out a determination of blood, liver, and kidney esterase in the same mice at the end of the excretion studies, to get a clearer picture of the metabolism of the enzyme. The following method was devised, as we were unaware of any quantitative studies on enzyme metabolism in small animals.

Excretion in urine.—Preliminary experiments had shown that the esterase activity of the urine diminished to a considerable extent on standing. Even collection under paraffin gave variable results. Twenty-four hour specimens were therefore unsuitable for this purpose, and it was decided to study a sample obtained during a shorter period of 6 hours and taken directly in a buffer and butyrate mixture. The following procedure was adopted: Three groups of 5 mice of each strain and of about the same age were kept in 3 different metabolism cages, which were fixed over large glass funnels. The mouths of the funnels fitted snugly into the base of the cages and the lower ends of their stems opened into graduated glass cylinders, which were kept for the collection of urine. The stems of the funnels

* Because of the difficulties of international communication the authors have not read proof of this article.

were closed by glass balls which permitted only the passage of urine. These glass cylinders contained 10 cc. of a phosphate buffer (pH 8.9) and 0.1 cc. of ethyl butyrate. The collection of urine was begun at 9 a.m. every day and was continued for 6 hours. During this period the mice were given only water. Food and milk were removed from the cages so that the urine should not be contaminated by them. At the end of the period the volume of urine was noted and the cylinders were incubated for a further 18 hours at 37.5° C. The liberated acid was titrated with N/10 NaOH. The result was multiplied by four to express the activity of 24 hours' collection. Necessary correction for the acidity of the collected urine was made by conducting a blank experiment in each case without the addition of the ester under identical conditions.

Excretion in feces.—Feces were collected for 24 hours from the funnels and the cages, and dried in a vacuum desiccator. The dried feces were weighed and powdered. They were then extracted with 20 cc. of 0.025 M ammonia for 6 hours. The extract was centrifuged and 2 cc., or 1/10th of the total amount of the supernatant liquid, was mixed with 10 cc. of phosphate buffer (pH 8.9) and 0.2 cc. of ethyl butyrate. The mixture was incubated for 18 hours, and the liberated acid was titrated with N/10 NaOH. The results were multiplied by 10 to represent the quantity in a 24 hour collection.

In Table II the excretion of esterase by these strains is recorded during a period of 10 days, and the data are statistically analyzed. In Table III the results of analysis of blood, liver, and kidneys of these animals are given. The kidney esterase was studied by the same method as that employed in the case of the liver.

TABLE I: LIVER ESTERASE

In terms of cubic centimeters of N/10 NaOH

C57 strain			C3H strain			A strain		
No.	Sex	NaOH per gm. of liver, cc.	No.	Sex	NaOH per gm. of liver, cc.	No.	Sex	NaOH per gm. of liver, cc.
159	♀	51.72	147	♀	35.24	135	♀	46.67
160	"	54.45	148	"	38.82	136	"	61.97
161	"	48.08	149	"	32.28	137	"	40.50
162	"	64.86	150	"	55.07	138	"	42.01
163	"	56.10	151	"	59.02	139	"	59.98
164	"	57.19	199	"	52.35	197	"	39.68
165	"	66.68	200	"	51.52	198	"	41.29
166	"	59.47	201	"	46.56	207	"	44.00
178	"	54.58	202	"	42.90	208	"	40.06
179	"	34.57	203	"	50.58	209	"	56.90
167	♂	33.96	152	♂	48.33	140	♂	52.84
168	"	44.16	153	"	38.11	141	"	40.00
169	"	47.68	154	"	33.96	142	"	38.90
170	"	55.72	155	"	47.97	143	"	43.48
171	"	53.06	156	"	55.34	144	"	36.15
172	"	48.39	157	"	35.89	145	"	36.31
173	"	46.49	158	"	50.70	146	"	50.14
174	"	35.84	180	"	73.62	194	"	46.40
175	"	47.32	181	"	62.23	195	"	45.19
176	"	45.08	182	"	50.58	196	"	38.46
177	"	46.67	183	"	45.21	206	"	50.00
Maximum		66.68			73.62			61.97
Minimum		33.96			32.28			36.15
Mean		50.10			47.92			45.28
Standard error..		± 1.99			± 2.18			± 1.57

DISCUSSION

A study of the tables shows that the mean esterase activity of the livers of C57 strain is 50.1 ± 1.99, of C3H strain 47.92 ± 2.18, and of A strain 45.28 ± 1.57. These values do not vary significantly from one another and it can be assumed that the livers of these strains manufacture the same amount of esterase. When this finding is compared with the difference in the blood serum one is led to believe that the livers of these strains do not differ in their capacity to manufacture this enzyme but probably in their ability to maintain an equivalent level in the blood. The capacity of the liver to regulate the blood level of certain essential metabolic products has been suggested by Soskin and his collaborators (7) in the case of glucose. They arrived at this conclusion on the basis of their experi-

ments on depancreatized and hepatectomized dogs. It may be assumed that the liver plays a similar and an important role not only in the preservation of a certain level of glucose in blood but also of other metabolites. The results of excretion of esterase in Table II indicate the mechanism by which the different blood levels are maintained in these strains. The urinary excretion is almost the same. The fecal excretion and consequently the total excretion, however, show a large difference. The C57 strain, which is insusceptible to cancer, excretes larger amounts (52.96 ± 2.99) than the two susceptible strains ($C3H = 17.14 \pm 0.177$, $A = 17.49 \pm 1.06$). This difference is statistically significant. The esterase activity of the kidneys, which is equal in all three strains, suggests that probably there is no difference in the renal excretory function.

TABLE II: URINE AND FECAL EXCRETION OF ESTERASE

In terms of cubic centimeters of N/10 NaOH

No. of days	C57			C3H			A		
	Urine	Feces	Total	Urine	Feces	Total	Urine	Feces	Total
1	2.11	59.70	61.81	1.69	13.50	15.19	1.72	14.70	16.42
2	3.91	44.00	47.91	2.77	16.00	18.77	2.72	15.00	17.72
3	1.51	32.00	33.51	1.97	16.00	17.97	1.88	15.00	16.88
4	1.95	55.90	57.85	2.49	15.00	17.49	1.84	13.80	15.64
5	2.65	56.00	58.65	2.53	13.50	16.03	1.64	15.00	16.64
6	2.99	58.50	61.49	2.21	16.00	18.21	2.92	16.50	19.42
7	2.07	56.00	58.07	1.49	15.00	16.49	1.76	18.00	19.76
8	2.91	44.00	46.91	3.09	13.50	16.59	2.80	14.50	17.30
9	2.43	44.50	46.93	2.25	15.50	17.75	2.52	16.50	19.02
10	2.43	54.00	56.43	2.93	14.00	16.93	2.12	14.00	16.12
Mean	2.49	50.46	52.96	2.34	14.80	17.14	2.19	15.30	17.49
Standard error	± 0.22	± 2.98	± 2.99	± 0.11	± 0.35	± 0.17	± 0.16	± 0.87	± 1.06
	Mice Nos... 211 to 215			Mice Nos... 216 to 220			Mice Nos... 221 to 225		

TABLE III: ESTERASE ACTIVITY IN TISSUES

No.	N/100 NaOH serum, cc.	N/10 liver, cc.	N/10 NaOH kidney, cc.
	C57 STRAIN		
211	20.00	40.00	10.00
212	18.50	48.39	9.50
213	19.00	46.66	7.30
214	16.00	45.00	10.50
215	16.00	46.66	9.50
Mean	17.90	45.34	9.36
Standard error	± 0.90	± 1.58	± 0.81
	C3H STRAIN		
216	33.50	42.85	7.80
217	35.50	56.62	9.00
218	30.50	46.24	10.00
219	35.00	46.15	11.09
220	27.00	45.00	10.30
Mean	32.30	47.37	9.63
Standard error	± 1.88	± 2.66	± 0.68
	A STRAIN		
221	34.00	48.00	7.80
222	37.00	39.10	9.44
223	28.00	47.60	12.50
224	29.50	45.20	9.70
225	31.50	46.00	10.04
Mean	32.00	45.18	9.89
Standard error	± 1.88	± 1.71	± 0.99

The question whether the high level of blood esterase and its lesser excretion are in any way related to the higher incidence of mammary cancer in the two strains of mice, remains problematical. The enzyme under investigation is a lipase, and it might be imagined that a continued higher level of a lipase in blood may indicate an abnormal fat and sterol metabolism, which may eventually contribute an endogenous factor necessary for an atypical cell proliferation of the mammary glandular tissue. Bergmann (1) has suggested a biological relationship between cholesterol and carcinogenesis, and several recent publications indicate that endogenous substances may play a role in the production of cancer, and could be demonstrated in the liver. Investigations of Selle, Brindley, and Spies (6) regarding the induction of malignant growth in mice after transplantation of freshly excised liver tissue, which did not show histological abnormalities and was free from metastasis, favor the belief that the activity of liver is probably involved in the process which leads from normal to cancerous growth. The findings of Greenstein and his associates (3) regarding the decrease of liver catalase in mice bearing transplanted tumors lends further support to this view. The present investigation also contributes to the same chain of ideas by suggesting a difference in the liver metabolism in cancer susceptible and cancer insusceptible strains.

SUMMARY AND CONCLUSIONS

The esterase content of the liver and the excretion of this enzyme have been studied in two strains of mice susceptible to mammary cancer (C3H and A) and in one insusceptible strain (C57).

1. The livers of these strains were found to be equally potent as regards this enzyme activity.

2. The results of excretion showed a large difference; the urinary excretion was almost equal but the fecal excretion, and consequently the total excretion, was higher in the insusceptible strain (52.96 ± 2.99) than in the susceptible strains ($C3H = 17.14 \pm 0.177$, and $A = 17.49 \pm 1.06$).

3. The serum esterase, as reported earlier, was higher in the susceptible strains than in the insusceptible strain.

4. No difference was noted in the kidney esterase, suggesting that probably there was no difference in renal excretory function.

5. The results indicate that the livers of these strains do not differ in their capacity to manufacture the enzyme, but probably do vary in their ability to maintain an equivalent level in the blood.

6. A probable influence of liver metabolism on malignant growth has been discussed.

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Testicular Tumors in Mice of Several Strains Receiving Triphenylethylene*

W. U. Gardner, Ph.D.

(From the Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

(Received for publication September 3, 1942)

The extensive use of mice in experiments undertaken to determine the possible role of steroid hormones in mammary carcinogenesis has resulted in a number of unanticipated observations, one of which was the appearance among some mice of an hypertrophy of the Leydig cells of the testes (5, 6, 10, 11). Burrows (7) noted that one mouse with a testis composed largely of such cells had apparently developed resistance to the injected estrogen. This was probably the first experimentally induced testicular tumor to be described, although "large circumscribed areas of interstitial cells" had been mentioned in another investigation (11).

Further studies identified these nodular areas as early stages of tumors of the interstitial cells, some of which could invade adjacent and remote tissues (3, 4, 15, 16, 22). These growths were described in mice of two different strains (A and C) that had received either stilbestrol (15, 16, 22), estradiol benzoate (15, 16), or triphenylethylene (3, 4).

Testicular neoplasms have appeared frequently among mice of the Strong JK strain given triphenylethylene. These mice have a very low incidence of spontaneous mammary tumors (24, 25) and tolerate large doses of estrogen very poorly (12).

The testicular tumors arising in the JK, A, and C3H mice and the response of the testes of mice of these and other strains to triphenylethylene will be described.

MATERIALS AND METHODS

Male mice of 6 different inbred strains were given weekly injections of 2.5 or 5 mgm. of triphenylethylene.[1] The compound was dissolved in sesame oil, either 50 or 100 mgm. per cc., and 0.05 cc. administered subcutaneously (Table I).

The mice[2] were from 33 to 91 days of age at the time the injections were started. They were autopsied as soon after death as possible, or killed when their condition indicated that death was imminent, or when large tumors appeared. The mice were given a commercial food mixture (Purina fox chow) and water.

The mammary glands, testes, seminal vesicles, prostates, and adrenals were fixed in Bouin's fluid. The mammary glands were dissected out for examination as whole mounts, and the other tissues, as well as parts of the mammary glands, were studied microscopically.

Dry imprints of several of the testicular tumors in this and other experiments were stained with the May-Grünwald-Giemsa stain. One growth was transplanted subcutaneously into the axilla of other mice of the same strain by inserting small pieces of tissue through a trocar. The mice bearing the transplanted neoplasms received weekly injections of 0.25 mgm. stilbestrol, or stilbestrol-cholestrol[3] pellets (1 part stilbestrol to 3 parts cholesterol).

OBSERVATIONS

Incidence of testicular tumors.—Of the 13 JK mice that survived 200 days or more, 7 had neoplasms of one testis. Only lesions resulting in an increase in the mass of the testis exceeding that of an untreated mouse were diagnosed as tumors. The mice with tumors survived periods of treatment for 314 to 398 days and those without them died after 249 to 330 days of treatment. All the mice of this group had received 5 mgm. of triphenylethylene weekly in one injection.

Eight of 17 mice belonging to the A strain died with testicular growths after periods of treatment running from 296 to 403 days. Of 14 mice of the C3H strain, a testicular tumor appeared in one, which had received 5 mgm. of triphenylethylene weekly for 652 days. Others dying without testicular neoplasms survived from 288 to 639 days. Tumors of the testes were not

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[1] The triphenylethylene was supplied in part by Dr. J. A. Morrell of E. R. Squibb & Sons Biological Laboratories and in part prepared under the direction of Professor W. Bergmann at the Sterling Chemistry Laboratory, Yale University.

[2] The mice, or the breeding stock from which they were derived, were furnished by Dr. L. C. Strong.

[3] The stilbestrol was supplied by Dr. J. A. Morrell of E. R. Squibb & Sons, New Brunswick, N. J.

found among mice of the C12I, CBA, and N strains (Table I).

The gross appearance of the tumors was similar in all strains. The smaller ones altered the shape of the testes, tending to make them spherical, were usually of a yellow or tan color, and were well vascularized. The larger growths were very irregular in shape and mottled in appearance—fleshy, deep brown, with yellowish areas occurring irregularly. Hemorrhage and necrosis were not uncommon in large tumors (Figs. 2 and 7).

In addition to the larger growths, which were recorded as tumors, small nodules or diffuse overgrowths of interstitial cells were noted in several testes (Figs. 1, 3). Since these have been noted earlier (11) and described in relation to blastomatogenesis of the testes in mice of the A (3, 4, 16) and C (22) strains, they will not be considered here. The "pretumorous" reactions of the interstitial cells observed in the JK mice (Figs. 1, 3) were similar to those of the A mice, with a few exceptions to be mentioned later. The testes containing the small nodular masses (Fig. 1) or the more diffusely distributed interstitial tissue (Fig. 3) were of approximately normal size as a rule and yellow or brown in color.

The testes in the C3H, CBA, N, and C12I strains, with very few exceptions, were reduced to less than one-half their usual dimensions and were either grayish or brownish in color. In the gross, they resembled those previously described in estrogen-treated mice of strains showing no appreciable interstitial cell hypertrophy (11).

Microscopic appearance of the testes and testicular tumors.—The testicular tumors of the A mice, which have already been described in detail (3, 4, 16), consisted predominantly of large Leydig cells with granular, eosinophilic, and vacuolated or spongy cytoplasm. Cysts, usually containing a clear fluid, were most prominent in the larger growths (Fig. 7). A few macrophages and brownish giant cells were found in some tumors, especially toward the periphery, and areas showing hematopoietic activity were observed in three of the growths from mice of the A strain.

The testicular tumors in the JK mice were composed largely of the same types of cells as those of the A mice. All the growths, even the smallest, only 2 or 3 times larger than the nodule shown in Fig. 1, contained islands of hematopoietic tissue that blended peripherally with the interstitial cells. Some of the small islands showed erythropoiesis at all stages of development from erythroblasts (Figs. 4 to 6). Other nodules contained a preponderance of granulocytes at different developmental stages (Fig. 5) as well as megakaryocytes (Fig. 4). These areas of erythropoietic or myeloid tissue were not distinctly separated from the surrounding neoplastic tissue. Hematopoietic foci were not observed in the nontumorous testes although these contained many Leydig cells, macrophages, and "brown cells."

TABLE I: TUMORS IN MICE OF 6 INBRED STRAINS THAT RECEIVED WEEKLY SUBCUTANEOUS INJECTIONS OF TRIPHENYLETHYLENE

Strain	No.	Ages started days	Ages autopsied days	Number of tumors *			
				Mammary adenocarcinoma	Lymphoid	Hepatic	Testicular
JK	13	33–91	250–478	—	—	—	7
A	17	48–79	357–467	1	—	—	7
C3H	14	20–45	288–672	10	1	4	1
C12I	6	46	340–384	—	—	—	—
CBA	5	34–58	545–758	1	—	1	—
N	3	55	396–641	—	1	—	—

* Lymphoid tumors and hepatomas: These appear in some untreated mice and their incidence was probably not altered by the treatment.

In the JK strain the tumors were all unilateral. The microscopic structure of the nontumorous testes of the different animals varied. Some showed a few spermatozoa or spermatids, although in most tubules spermatogenesis had been stopped at the primary spermatocyte stage. The interstitial tissue was either nearly normal in appearance, consisting of large Leydig cells with spongy cytoplasm and a vascular stroma, or was relatively more abundant than normal and contained lightly staining Leydig cells, large brown cells or masses, mast cells, and macrophages. The testes in which the tubules were nearly normal were of approximately normal size, consistency, and color, whereas those with damaged tubules and increased interstitial tissue were smaller than normal and invariably pale brown or yellow. The mediastinal and peripheral seminiferous tubules were most degenerated.

The one testicular tumor in a C3H mouse differed morphologically from all the others (Fig. 10). The malignant cells were arranged in small cords or alveolar structures, were small, and had a granular cytoplasm (Fig. 11). Large cysts or diffuse fluid-filled spaces in which strands of malignant cells were grow-

ing were found throughout the tumor. Malignant epithelial cells were found in several abdominal lymph nodes, the spleen, and the lungs. The comparatively undifferentiated condition of the cells in this tumor was associated with a very diffuse metastatic growth in the lymphoid tissues. The invasive cells were not arranged in masses, as previously described (15) for other testicular neoplasms, but lay singly or scattered in thin and irregular strands.

With these exceptions the testes of the mice from the C3H, CBA, N, and C12I strains resembled those previously described for mice of strains not showing testicular tumors (11). The small seminiferous tubules were closely packed together in the small testes. Spermatogenesis had usually stopped at the primary spermatocyte stage, although areas in many tubules contained few germinal cells. The interstitial tissue was relatively no more abundant than in the normal testes of untreated animals, and true Leydig cells with either granular or spongy cytoplasm were rarely found. Fibroblasts, large brown cells or polynuclear masses, and blood vessels occupied the greater part of the available intertubular spaces (Fig. 9). The testes of 3 mice, 2 CBA's and 1 C3H, had an increased amount of interstitial tissue. In these 3 animals the brown cells were abundant and were accompanied by large Leydig cells with pale staining, spongy cytoplasm.

The pituitary glands of all the mice were slightly larger than normal, but none was greatly enlarged. The adrenal glands of all the mice of the JK, C3H, and CBA strains showed definite perimedullary degeneration. Brown cells or polynuclear masses completely surrounded the medulla. An amyloid degeneration of the same adrenal zone occurred in mice of the A strain (2).

The incidence of mammary tumors was very high in the C3H strain (Table I). Although mice of the A strain also show a high incidence of spontaneous mammary tumors, only one was found among the 17 receiving triphenylethylene. Few or no mammary tumors appeared in the CBA, C12I, N, or JK strains. The mammary glands had developed in all groups. In the C3H, A, and CBA strains nodules of glandular tissue were frequently seen in otherwise atrophic glands. The glands of the JK and N mice showed a uniform structure throughout. The amount of mammary alveolar development was always most abundant in the glands of the low tumor JK strain.

Transplantation experiments.—One of the tumors in a mouse of the A strain that had received 265 mgm. of triphenylethylene in 376 days, starting at an age of 61 days, was grafted into 2 other mice of this strain. The hosts received weekly injections of 0.25 mgm. of stilbestrol. The tumor grew in both animals, reaching a size of 1.2 × 1.5 cm. after 3.5 months, and has been carried through 4 transfers up to the present time. It has grown in all except 2 of 37 estrogen-treated hosts, but has not grown progressively in any of the 10 hosts that received weekly injections of sesame oil, pellets of cholesterol, or no treatment at all. When the transplanted tumors of the second transfer generation had attained a diameter of approximately 1 cm., after 10 weekly injections of stilbestrol, these were discontinued in 2 of 4 mice; yet the tumors grew progressively, and when the hosts were killed 3 months later had invaded the skin and were almost the same size as those in the estrogen-treated controls.

Three of 6 mice bearing the third transplant generation of the testicular tumor, together with pellets of stilbestrol and cholesterol, were hypophysectomized 42 days after transplantation. The transplanted tumors almost doubled in volume within 2 weeks after the operation and compared in size with those of intact controls, although the hosts had lost 3 to 4 gm. in total body weight.

DISCUSSION

The appearance of tumors of the testicular interstitial cells among mice of several different strains after the injection of estrogens, and not in those of other strains, indicates the existence of factors or influences that are strain-limited. These growths have arisen in the A, C, and now in the JK strain, with a sufficiently high incidence to set these strains apart from others

DESCRIPTION OF FIGURES 1 TO 5

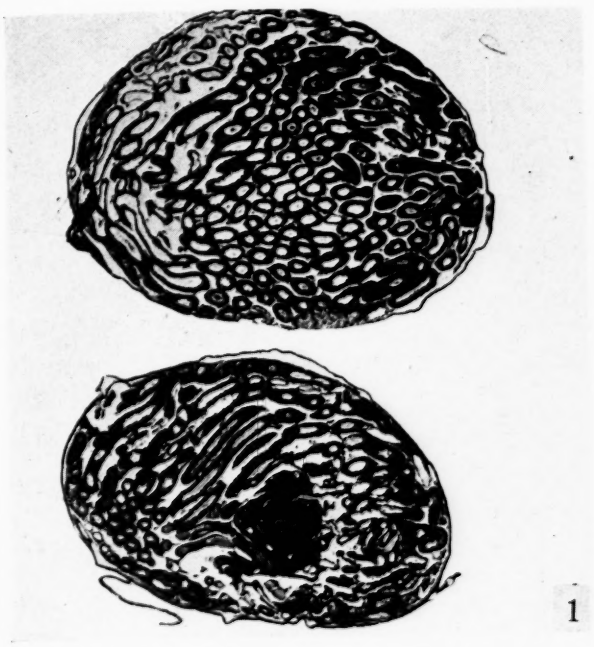
FIG. 1.—Cross section of the testes from a JK mouse which had received 280 mgm. of triphenylethylene in 398 days, starting at 80 days. The testes were of nearly normal size and light yellow in color. Some tubules showed complete spermatogenesis. A nodule of Leydig cells was found in one testis. Mag. × 7.5.

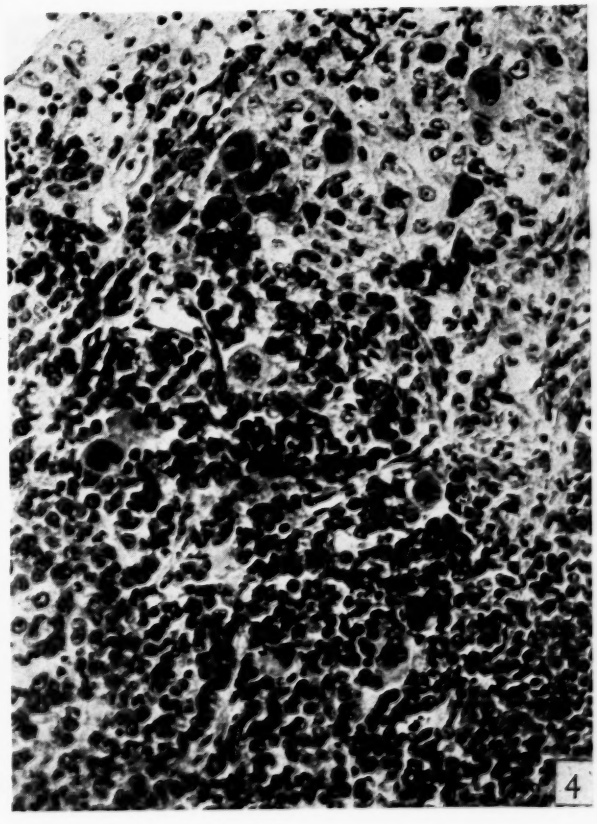
FIG. 2.—Section of a testis from a JK mouse that had received 230 mgm. of triphenylethylene in 323 days, starting at 70 days. The tumor, which had largely replaced the tubules and greatly enlarged the testis, was composed of Leydig cells and areas of hematopoietic tissue. Mag. × 7.5.

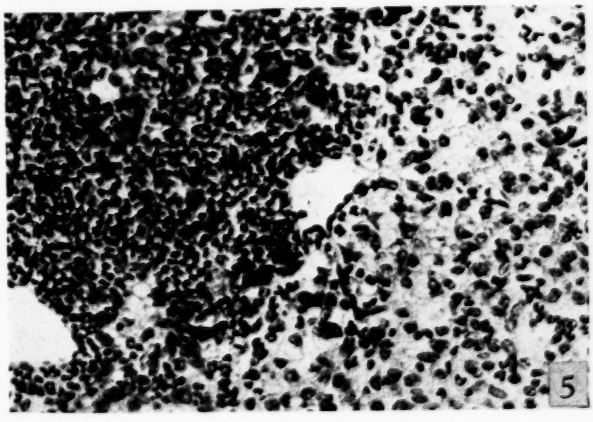
FIG. 3.—Testis of a JK mouse that had received triphenylethylene for 249 days. Beneath the tunica albuginea a group of interstitial cells widely separated the damaged tubules. Mag. × 112.

FIG. 4.—Large testicular tumor in a JK mouse that had received 260 mgm. of triphenylethylene in 365 days. Leydig cells appear at the upper right. A group of erythroblasts and normoblasts and a few megacaryocytes are shown below. Mag. × 224.

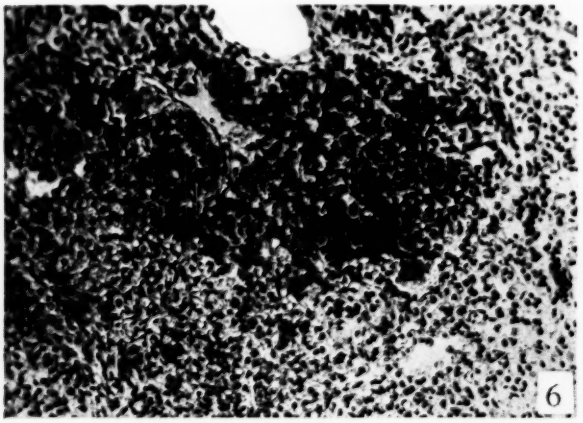
FIG. 5.—Testicular tumor of a JK mouse that had received 270 mgm. of triphenylethylene in 382 days, starting at 91 days. Among the interstitial cells areas of myeloid tissue showing both erythropoiesis and granulocytopoiesis were found. Mag. × 112.

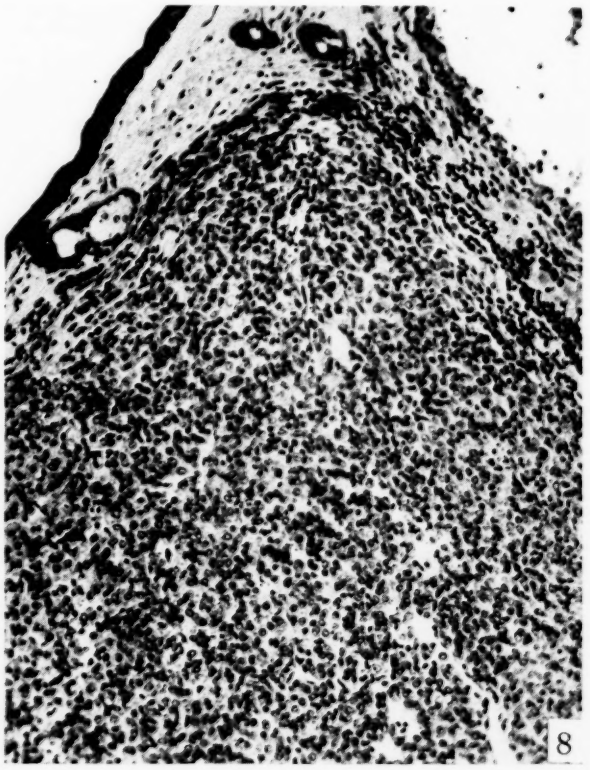






FIGS. 1-5





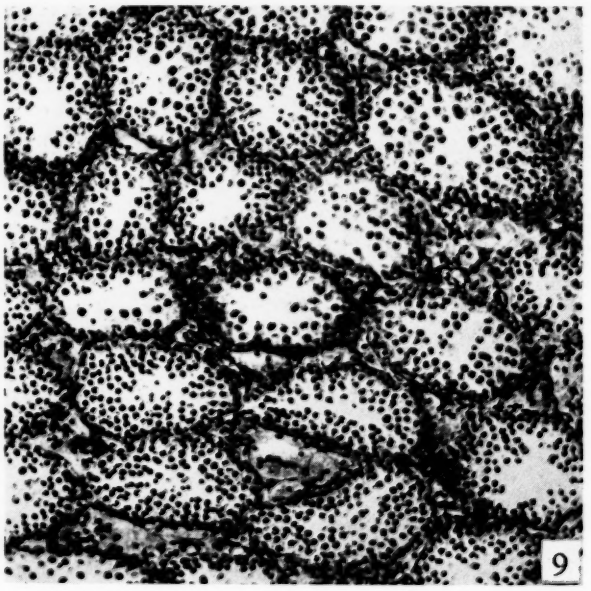


FIG. 6.—Another area from the tumor of the same mouse showing an erythropoietic area. Mag. × 112.

FIG. 7.—Testicular tumor of an A mouse that had received 265 mgm. of triphenylethylene in 376 days, starting at 61 days. The large growth contained several clear cystic and hemorrhagic areas. Remnants of tubules were found among the interstitial cells. Mag. × 7.5.

FIG. 8.—Transplant of the tumor shown in Fig. 7 growing subcutaneously in an estrogen-treated A mouse. The cells were the same as the predominant type in the original growth (Fig. 7). Mag. × 112.

FIG. 9.—Testis of a C3H strain mouse that had received 300 mgm. of triphenylethylene in 464 days, starting at 42 days. The testes were approximately one-half normal size and brown in color. The damaged tubules were separated by scattered large cells or multinuclear cells or masses. In 2 mice of this strain somewhat larger amounts of interstitial tissue were found. Mag. × 112.

such as CBA, C3H, C12I, N, F, or RIII (3,11,22). The A, C, and JK strains might be considered to possess some common genetic factor or nongenetic influence especially predisposing them to this response to estrogens.

The strain-limited neoplastic response of mice to estrogens occurs with reference to mammary (12) and pituitary tumors (14), as well as to those of the testis. The mammary tumors depend upon both genetic and nongenetic influences (1). The nongenetic, or "milk influence," is probably of greatest significance in limiting mammary carcinogenesis to mice of certain strains.

Few hypophysial or testicular tumors appear in mice that have not received estrogens. Transmission of the tendency to hypophysial tumors is compatible with a genetic interpretation (13). A "milk influence" did not alter the testicular neoplastic response of estrogen-treated C mice or mice suckled by animals of the C strain (22).

The appearance of testicular tumors among mice that have received triphenylethylene (3, 4), stilbestrol (15, 22), estradiol benzoate (22), estradiol dipropionate, or pellets of estrone indicates that the different estrogenic chemicals elicit qualitatively comparable responses. This would suggest that the neoplasms do not arise because of specific chemical stimulation unassociated with the estrogenic quality of the substance.

On the other hand, testicular growths have not appeared so far in JK mice that have received other steroid estrogens or stilbestrol. Those of the CBA, C12I, N, C57, PM, and C3H strains have shown no testicular tumors when the naturally occurring estrogens or their esters were injected. One CBA and one C3H mouse that had received stilbestrol, however, had testicular tumors at death. Since these growths occurred only in CBA and C3H mice that had received the two synthetic estrogens, and have not appeared in a large series of male mice of these two strains that received estrone, estradiol, or their esters, these substances might be considered to have a special action on the testis (unpublished data). Much more work would have to be done, however, before the apparent differences could be attributed to other than quantitative factors.

The inhibitory action of sex hormones on the gonads and the decrease of hypophysial gonadotropin in estrogen-treated mice led to the theory of a reciprocal hypophysial-gonadal relationship (19). This theory was modified to some extent when it was shown that the luteinizing hormone (LH) content of the hypophyses of estrogen-treated rats was increased (17), an

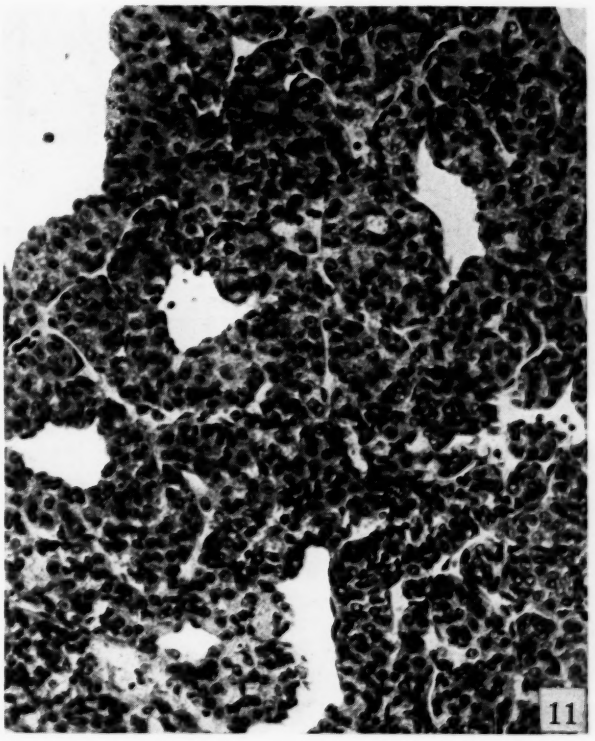


FIG. 10.—Testicular tumor in a C3H mouse that had received 465 mgm. of triphenylethylene in 652 days, starting at 20 days. Mag. × 7.5.

FIG. 11.—Tumor reproduced in Fig. 10, showing the peculiar arrangement of its cells. This growth had metastasized throughout the lumbar and renal nodes and the lungs. Mag. × 225.

indication that the estrogen might be eliciting the testicular responses through the hypophysis since LH and the hypophysial interstitial cell stimulating hormone appear to be identical (8, 9).

If it is assumed that the estrogens act indirectly upon the interstitial cells, and that the resulting effect is responsible for the neoplasia, then either the hypophyses or the testes must differ in the several strains. This assumption might be tested directly by injecting extrinsic hypophysial hormones into mice of various strains and determining the comparative response, or by bioassay of hypophyses from estrogen-treated mice of the different strains. The injection of a gonadotropic hormone increased the amount of interstitial tissue of male mice (20).

The relation of the Leydig cells to fibroblasts on the one hand and to macrophages on the other, has been a subject of controversy. Most investigators apparently agree that the Leydig cells do not store acid vital dyes unless these are injected in extremely large amounts (23). The apparent evolution of Leydig cells from fibroblasts or fibroblast-like cells has been reported, and Maximow has stated that Leydig cells, in culture, revert to fibroblasts (18). Extramedullary myelopoiesis is not rare in mice, and its appearance in testicular tumors might be of unique interest as an indication that these structures contain tissue capable of differentiating in several directions.

The studies on the transplanted tumors, although affording no information concerning their origin, show their capacity for continued growth under several conditions. Some grow in all mice of the same strain while others grow in males but not in females (26), in aged but not in young animals (21). Transplanted testicular tumors have grown when their hosts had received estrogens (16), but after those of the second transfer generation started to grow, subsequent to estrogen-treatment for a period of 10 weeks, they continued their progressive course in the absence of further treatment, as shown in the present experiments. Transplanted tumors continued to proliferate in hypophysectomized animals. Estrogens, either directly or indirectly, facilitated the establishment of transplanted malignant testicular tissue, but after growth had started either it was capable of an independent existence or the estrogenic effects induced in the hosts were not reversible.

SUMMARY

Interstitial cell testicular tumors appeared in 7 of 13 mice of the JK strain that had received weekly subcutaneous injections of 5 mgm. of triphenylethylene for 250 days or more.

Seven of 17 mice of the A strain similarly treated had interstitial cell tumors, one of which has been transplanted for 4 generations. Although it has grown progressively only in mice that received estrogens for 10 weeks, it grew for 3 months after the last injection and has continued to grow after hypophysectomy.

One of 14 male C3H mice that received triphenylethylene died with a testicular growth that had metastasized to lymph nodes, spleen, and lungs.

Tumors of the testes were not found in mice of the C12I, N, or CBA strains similarly treated. The interstitial tissue was present in increased amounts in only 3 mice of these strains, where it consisted of large, clear Leydig cells and brown cells. Brownish cells and fibroblastic elements filled the small intercellular spaces in the other mice.

Hematopoietic foci occurred in the testicular tumors of all the JK mice, and of 3 A mice. Some islands of hematopoietic tissues contained largely erythroblasts and normoblasts, while others showed developing granulocytes and megakaryocytes.

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The Possible Carcinogenicity of Overcooked Meats, Heated Cholesterol, Acrolein, and Heated Sesame Oil*

Paul E. Steiner, M.D., Robert Steele, Ph.D., and F. C. Koch, Ph.D.

(From the Departments of Pathology and of Biochemistry, The University of Chicago, Chicago, Ill.)

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INTRODUCTION

This paper is concerned with studies of the carcinogenicity of several heated substances, in a preliminary survey of certain dietary constituents as possible extrinsic, environmental causes of cancer.

The cause of the high incidence of cancer of the alimentary tract, particularly of the stomach and colon, in Europeans and Americans remains unexplained. There is also no adequate explanation for the comparatively low incidence of these cancers in certain primitive peoples, notably the Bantu of the East Coast and of South Africa (5, 6, 27, 37), and the Javanese (8, 9), whereas both these races are subject to much primary liver cancer. Even more striking is the observation that in contrast to the low incidence of primary cancer of the stomach and large bowel and the high incidence of hepatic cancer in the African Bantu, the incidence at these two sites is reversed in the Afro-American, whose distribution of these cancers comes to resemble that of whites in America (21, 17). While negroes in America are not all Bantus, surely some of them are (21). The comparatively sudden change in incidence of these different types of primary cancer suggests that environmental rather than hereditary factors may be playing the dominant role in their causation.

This assumption is supported by another line of evidence; namely, that obtained from the examination of negro livers for a carcinogen. Thus the noncancerous livers of the African Bantu commonly contain a carcinogen according to des Ligneris (14), those of the Afro-American only rarely (34).

If cancers of the human alimentary tract are induced by exogenous carcinogens derived from the environment, the portal of entry would probably be the mouth, and the carcinogenic factor would almost of necessity be carried by food or drink. It is known from the work of Lorenz and Stewart that carcinogenic hydrocarbons taken by mouth can cause cancer of the stomach (22) and of the intestine (23) in mice. It is also known that the diet of the South African Bantu is different from that of the Afro-American, and that the Javanese eat food different from that of other races living in Java which have an entirely different tumor incidence (8).

From what is known about the chemistry of the synthetic carcinogens (13, 16) one of the most probable dietary sources of carcinogens would be meats or meat products, or vegetable oils, which had been subjected to high temperatures in cooking. Indeed, there are numerous reports which state that such products have carcinogenic activity for animals.

In testing food products for carcinogenicity rats and mice have been used almost exclusively. In evaluating the outcome of experiments it is important to know that these species react differently. Rats on the whole have been much more susceptible than mice to tumor induction by injection and by feeding. Little is known about the reaction of rats to skin painting by these foodstuffs, while mice, according to the few experiments in which they were painted, appear to have no unusual degree of susceptibility.

A great amount of work has been done, since the report by Singer in 1913 (32), on the production of gastric lesions in rats by various diets that are deficient in total caloric value, in certain of the principal food constituents, or in vitamins. In general, the lesions develop quickly, and consist of ulcers or papillomas with the occasional production of infiltrative lesions later. Since this paper is not primarily concerned with unbalanced diets, reference is made to recent papers and reviews by Morris and Lippincott (25), Beck and Peacock (4), Brunschwig and Rasmussen (11), and Klein and Palmer (20), where details may be found.

Of greater immediate interest are the various animal and vegetable substances that have been tested for carcinogenicity either because of direct interest in the problem as related to foodstuffs, or because they were used as solvents in other experiments. Kennaway and Sampson in 1928 (18) painted 70 mice twice weekly with the tar obtained by heating cholesterol to 800–

* This investigation was aided by grants from the National Advisory Cancer Council and the Commonwealth Fund.

810° C. The first tumor appeared on the 83rd day. The total number was 18, of which 11 were considered malignant. Three years earlier Kennaway (19) had made cancer-producing tars from human skin and from yeast by heating to 920° C.

Barry and Cook (2) stated that injection of lard elicited granulomas, but not tumors, in 2 out of 10 rats, and that it produced no tumors in 20 mice, of which the longest survival was 581 days. Andervont (1) injected mice with a total of 1 cc. of lard, given in 3 injections in 3 months, and induced no tumors in 190 mice in 189 days. Burrows, Hieger, and Kennaway (12) summarized numerous experiments involving the injection of various oils and fats. In 236 mice no tumors arose, but in rats the injection of lard was followed by 7 sarcomas in 134 animals, and 1 sarcoma was induced by olive oil plus arsenious acid. Lard heated to 340–360° C. was also not carcinogenic for 12 mice but induced 2 sarcomas in 14 rats.

Schabad (31) injected sunflower oil into 16 mice in quantities of 0.25 to 0.50 cc. on 3 occasions, but no tumors had appeared after 6¼ months. Recently, Beck (3) heated cottonseed oil to 340–360° C. for 1 hour in air, or repeatedly at 200–210° C. for a total of 12 hours. The first sample induced 2 sarcomas on subcutaneous injection in 0.5 cc. doses, at 414 and at 535 days, in 6 mice, while the latter elicited none. Rowntree and his coworkers (30) induced abdominal sarcomas by feeding rats a crude wheat germ oil. This work has not been confirmed by others (10).

Widmark (40) painted mice with alcohol or petroleum ether extracts of horse muscle heated to 275° C., browned butter, or roasted coffee, but no sarcomas were induced. Of 241 mice, 23 females survived for 11 months. Nine of them developed mammary gland tumors, an incidence of 40 per cent compared with a normal incidence of 10 per cent for this stock. Two of these tumors followed the petroleum ether extract of roasted coffee, 2 the similar extract of roasted muscle, 1 this extract of browned butter, and 4 the alcohol extract of roasted muscle. He also induced 1 sarcoma in a mouse painted with cholesterol treated at 275° C.

The strongest claims for the carcinogenic activity of heated foodstuffs have come from the laboratories of Roffo and of Waterman. In a series of papers Roffo (28, 29) described the induction of benign and malignant tumors in the stomach and liver in rats by feeding overheated cholesterol, and animal and vegetable fats. He used lard, veal fat, mutton fat, and olive oil, heated to 350° C. for 30 minutes. He thought that the cholesterol was oxidized to a carcinogenic chemical. Waterman (39) fed cholesterol oleate to mice and obtained malignant tumors in the stomach after about 1 year. Veldstra (38) thought that the active substance in cholesterol oleate was $\Delta^{3,5}$-cholestadiene. Domagk (15) described a tumor of the stomach in a mouse after prolonged feeding of a diet rich in olive oil. A critical analysis of the chemical work in these papers has been made by Cook and Kennaway (13), and of the pathology by Klein and Palmer (20).

EXPERIMENTAL

In presenting the experimental results tables are given to show the sex and the number of mice used in testing the various substances for carcinogenicity, the number surviving at various periods of time, and the number of tumors induced. These data are not repeated in the text, where only the method of preparation of the extracts, the details of dosage, the general reactions, and details respecting the tumors are given. All injections were made subcutaneously in the interscapular region.

It may be stated here that four sarcomas were induced in these experiments. Of these, one is described in experiment 4, and three in experiment 17.

The mice used in experiments 1 to 6 and 13 to 17 inclusive were from our own stock of partly inbred albinos. Their susceptibility to tumor induction and something about their incidence of spontaneous tumors have been described in several publications (33, 35, 36). They are susceptible to subcutaneous sarcoma induction but sarcomas do not arise in them spontaneously. In experiment 7 the mice used were first generation offspring of C57 brown breeding stock obtained from the Roscoe B. Jackson Memorial Laboratory. All others used in these experiments were purchased from dealers, and nothing is known about their susceptibility to tumors. The mice were usually about 3 months of age when the experiments were begun, although a few were younger and others older.

Carcinogenicity of Meat Extracts

Preparation of meat extracts used in experiments 1 to 7.—Twenty pounds of good grade beef, 20 pounds of good pork, and 5 pounds of suet were ground up together, mixed thoroughly, formed into small meat balls, and fried in an open pan. The frying was carried out to a crisp brown, somewhat past the point generally used in the preparation of human food, but without burning. The fat which drained away readily in the melted state was poured off. It weighed 3,280 gm. and was used without further change for injection in experiment 1 and for feeding in experiment 2.

A 300 gm. portion of the crude fat thus obtained was saponified with 1.5 liters of alcohol and 300 gm. of sodium hydroxide in 300 cc. of water for 1 week at room temperature. The mixture was then diluted with water and extracted 3 times with benzene. The ben-

zene extracts were pooled and washed 3 times with water, the emulsion being broken by alternate freezing and thawing. The benzene extract was finally evaporated to dryness, yielding 1.14 gm. of an oil. This was made up in sesame oil so that 0.5 cc. contained 0.1 gm. of extract. This was used for injection in experiment 3.

The fried meat was reground and extracted with 1 volume of acetone for 24 hours. This procedure was repeated twice. The meat residue was allowed to dry. The acetone extracts were combined and evaporated to a small volume, when an aqueous and a fatty phase separated. The aqueous phase was diluted again with acetone and reevaporated until no more acetone came off. A new fatty phase separated in sesame oil so that 0.5 cc. contained 0.15 gm. This was used for injection in experiment 7.

Experiment 1. Injection of fat from overfried mixed meats.—Twenty mice were injected each with 0.5 cc. of the warmed fat which had been poured from overfried meats. At intervals of 4 weeks the injections were repeated so that each mouse had a total of 5 injections within a period of 4 months. The total dose for each mouse was 2.5 cc.

This fat was well tolerated. It persisted in the form of soft masses for several months, but no mouse autopsied after 11 months showed any unabsorbed residue. See Table I for results.

Experiment 2. Feeding of fat from overfried meats.—Thirty mice were placed on the stock diet to

TABLE I: CARCINOGENICITY OF MEAT EXTRACTS FOR MICE

Experiment No.	Male mice	Female mice	Total mice	Survival time in months										Sarcomas induced
				6	9	12	15	18	21	24	27	30	33	
1		20	20	20	20	19	18	15	13	5	3	1	0	0
2		30	30	30	29	27	15	11	4	4	1	0		0
3		10	10	10	10	8	8	7	5	2	1	0		0
4		20	20	20	20	18	16	10	7	3	2	1	0	1
5		30	30	29	26	15 *	13	10	6	3	0			0
6		8	8	8	8	7	6	6	4	0				0
7	9		9	9	9	9	5	0						0
8 A	24	24	48	18	13	9	0							0
8 B		10	10	1	1	0								0
9	58	24	82	21	7	1	0							0
10	36	12	48	9	2	1	0							0
11		26	26	10	0									0
12 A		25	25	7	0									0
12 B		21	21	6	1	1	0							0

* Ten mice killed for gastric examination.

rated. The fatty phases were combined. This fat weighed 403 gm.

The dried meat residue (about 5,500 gm.) was extracted with benzene in continuous extractors. The tissue was ground in a Wiley mill and reextracted with benzene. The benzene extracts were combined, filtered, and evaporated to dryness. This fat weighed 1,770 gm. It was used for injection in experiment 4 and for feeding in experiment 5.

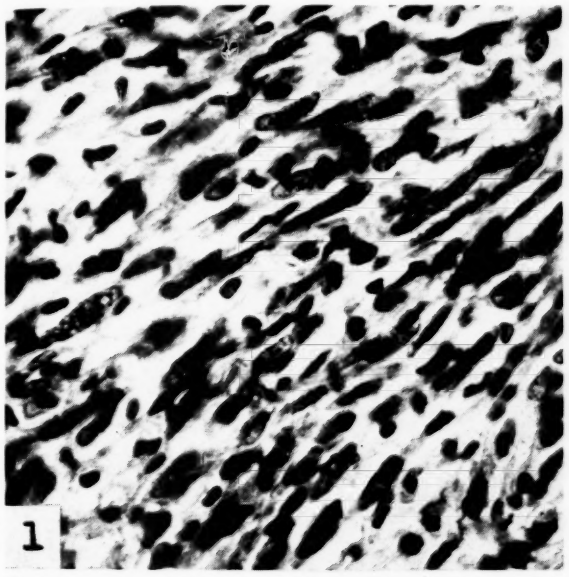
A 300 gm. portion of this benzene extract fat was saponified by the methods employed for the supernatant fat mentioned above. The yield of nonsaponifiable material was 0.94 gm. of an oil. It was made up in sesame oil so that 0.5 cc. contained 0.1 gm. of extract, and used for subcutaneous injection in experiment 6.

The fat from the acetone extraction (403 gm.) mentioned above was dissolved in benzene and extracted with 50 per cent alcohol. The benzene was evaporated to dryness, leaving 330 gm. of oily residue. Of this 300 gm. was saponified by the methods given above. The 2.0 gm. of nonsaponifiable material obtained was which 5 per cent by weight of this meat fat was added. For a period of about 3 weeks early in the course of the experiment the amount of fat added to the diet was 10 per cent. The mice thrived on this diet. Each consumed about 145 mgm. of fat daily for 14 months.

Experiment 3. Injection of the nonsaponifiable lipids from overfried mixed meats.—Ten mice were injected each with 100 mgm. of the nonsaponifiable lipids from the fat used in experiment 1, dissolved in 0.5 cc. of sesame oil. This material was well retained, and many mice showed traces of it when autopsied more than a year later. Each mouse received the nonsaponifiable lipids from 30 gm. of the original fat.

Experiment 4. Injection of a benzene extract from overfried mixed meats.—The fat obtained by benzene extraction was injected into 20 mice. Each was given 0.5 cc., followed at intervals of 4 weeks by injections of the same size. Each mouse had a total of 5 injections (2.5 cc. of fat) within a period of 4 months. This fat, which was well tolerated by the mice, per-

sisted longer than did the fat in experiment 1. At autopsy masses of it were seen as long as 18 months after the first injection.



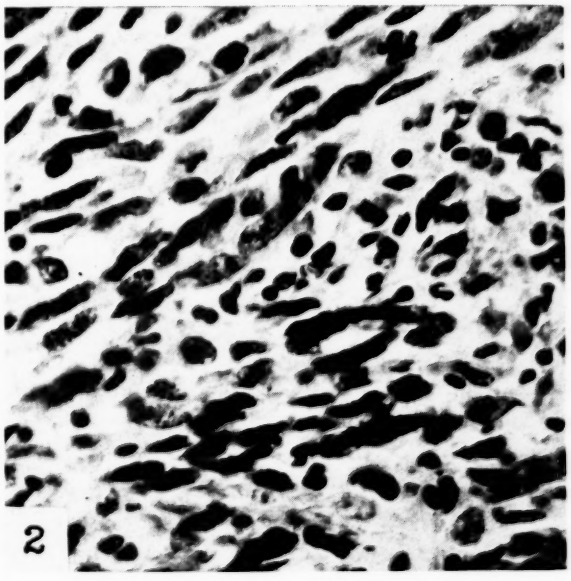


FIG. 1.—Spindle cell sarcoma induced in experiment 4 at the site of injection of a benzene extract from overfried mixed meats. Mag. × 420.

FIG. 2. Spindle cell sarcoma induced by heated sesame oil in the 20th month after the first injection. Mag. × 420.

One mouse developed a subcutaneous sarcoma in contact with a mass of the fat and died 424 days after the first injection. Besides a subcutaneous tumor 40 × 20 × 15 mm. in the right lateral chest region which had the gross and microscopic appearance of a mammary gland carcinoma, and 2 lung nodules each 2 mm. in diameter which microscopically proved to be primary lung tumors, this mouse had an oval subcutaneous mass 22 × 12 × 10 mm. located in the left groin. About one-third of this mass consisted of hardened white fat and the other two-thirds appeared like homogeneous pinkish-white tumor, which partly enclosed the fat. Upon microscopic examination it was found to be a spindle cell sarcoma. (See Fig. 1.)

Experiment 5. Feeding of a benzene extract from overfried mixed meats.—Thirty mice were placed on the stock diet to which 5 per cent by weight of this fat was added. For a period early in the experiment the amount of fat added was 10 per cent. The animals appeared to thrive on this mixture and it was fed until the supply was exhausted, at 330 days. Each mouse consumed about 145 mgm. of fat per day.

When the feeding of fat was discontinued 10 mice were sacrificed and their stomachs examined. They showed no recognizable lesions, nor did any of the others subsequently develop tumors of the alimentary tract.

Experiment 6. Injection of nonsaponifiable lipids of a benzene extract from overfried mixed meats.—Eight mice were injected each with 100 mgm. of this extract dissolved in 0.5 cc. sesame oil. Although it was retained no sarcomas developed. The 100 mgm. which each mouse received represented the nonsaponifiable lipids from 37.5 gm. of the original fat which had been used in experiments 4 and 5.

Experiment 7. Injection of the nonsaponifiable lipids from the benzene-soluble material of an acetone extract of overfried mixed meats.—Nine mice were each given 1 injection consisting of 150 mgm. of this extract in 0.5 cc. of sesame oil. This represents about 22.5 gm. of the original fat, which was fairly rich in nonsaponifiable material.

Preparation and Testing of Meat Extracts Used in Experiments 8 to 10

Ten pounds of fresh boiling beef, 10 pounds of fresh pork shoulder which was rather fatty, and 1 pound of suet were ground up together. Half of this was saponified with 450 gm. of potassium hydroxide and 2 liters of 95 per cent alcohol on a steam bath and the remainder divided into 2 equal portions, of which one was overfried and the other overbaked.

The meat to be fried was moulded into flat cakes and heated in an open pan until the surfaces were brown and crisp, although the inside was still soft. Saponification with potassium hydroxide was then carried out on a steam bath for 3 days, water being added.

The meat used for baking was moulded into a loaf and baked until the surface was brown and crisp, a point somewhat beyond that usually used for food. The temperature in the oven was between 185° and 220° C. for a period of 3½ hours. This meat was then also saponified.

After saponification the three meat portions—raw, fried, and baked—were separately extracted 5 times with ethylene dichloride, the extract was washed with water, dehydrated with anhydrous sodium sulfate, filtered, and distilled to small volume in partial vacuum. The oily residues were resaponified with alcoholic potassium hydroxide, and the final nonsaponifiable extracts obtained were: Raw meat, 3.1 gm.; fried meat, 3.1 gm.; baked meat, 2.8 gm. These extracts were made up in sesame oil so that 0.1 cc. contained 25 mgm. of the extract and tested for carcinogenic activity by subcutaneous injection into mice (experiments 8, 9, and 10).

Experiment 8 A. *Injection of raw mixed meat extract.*—Forty-eight mice were given 1 injection consisting of 25 mgm. of the nonsaponifiable lipids in 0.1 cc. of sesame oil. When this induced no tumors a larger amount was tried (Experiment 8 B).

Experiment 8 B. *Injection of raw mixed meat extract.*—In this experiment 100 mgm. of nonsaponifiable lipids used in experiment 8 A was dissolved in 0.2 cc. of sesame oil and injected into each of 10 mice.

Experiment 9. Injection of fried mixed meat extract.—Eighty-two mice were injected with 25 mgm. of this nonsaponifiable lipid extract in 0.1 cc. of sesame oil.

Experiment 10. Injection of baked mixed meat extract.—Forty-eight mice were injected with 25 mgm. of this extract dissolved in 0.1 cc. of sesame oil.

Experiment 11. Nonsaponifiable lipid extract of beef.—This extract and that used in experiment 12 were prepared by Dr. Carl Marberg, who kindly gave his permission to include this work. Ten pounds of fresh beef of average fatness was ground and made into meat balls. These were fried until they were well done—hard and brown, but not burned. The meat, together with the fat, was transferred to a flask and treated with an alcoholic solution of potassium hydroxide. After 2 hours a volume of water equal to that of the alcohol was added and the material was refluxed on the steam bath overnight. The next day the nonsaponifiable material was extracted 5 times with ethylene dichloride. The ethylene dichloride was evaporated *in vacuo,* and the residue was resaponified with alcoholic potassium hydroxide. After dilution with water the soap solution was again extracted with ethylene dichloride, and the combined extracts were dried with sodium sulfate and evaporated *in vacuo.* The 3.0 gm. of residue was dissolved in sesame oil. Twenty-six mice were given 1 injection of 30 mgm. of this extract in 0.1 cc. of sesame oil.

Experiment 12. Nonsaponifiable lipid extract of mixed meats.—A mixture of equal parts of beef, pork, and lamb with a total weight of 10 pounds was treated exactly as was the beef in experiment 11. The final nonsaponifiable extract weighed 3.6 gm. In experiment 12 A 26 mice were each given 1 injection of about 36 mgm. of this extract dissolved in 0.1 cc. of sesame oil. When this induced no tumors the same extract was used in doses 3 times as large; namely, 108 mgm. per mouse (experiment 12 B).

TABLE II: CARCINOGENICITY OF MISCELLANEOUS SUBSTANCES

Experiment No.	Substance tested	Male mice	Female mice	Total mice	Survival time in months							Sarcomas induced
					6	9	12	15	18	21	24	
13	Unheated cholesterol	18		18	12	9	7	2	0			0
14	Cholesterol, 200° C.	20		20	18	8	2	1	0			0
15	Cholesterol, 300° C.	14	16	30	16	10	6	3	2	0		0
16	Acrolein		15	15	14	14	11	6	3	1	0	0
17	Heated sesame oil	16	15	31	26	15	9	6	4	1	0	3

PREPARATION AND TESTING OF THE CHOLESTEROL USED IN EXPERIMENTS 13, 14, AND 15

The cholesterol for experiment 13 was freed from 7-dehydrocholesterol and impurities causing general absorption in the ultraviolet range by treatment with bromine according to the method of Bills, Honeywell, and MacNair (7). Ten grams of the purified cholesterol was incorporated in 100 cc. sesame oil. For experiment 14, 10 gm. of the purified cholesterol was heated in a 25 × 200 mm. open tube at 200° C. for 2 hours by immersion in an oil bath. After cooling the mass was dissolved in 95 per cent ethanol, transferred to 100 cc. sesame oil, and the alcohol removed by evaporation under diminished pressure. For experiment 15 the same procedure was followed, but at 300° C.

Experiment 13. Unheated cholesterol.—Eighteen mice were injected each with 50 mgm. of cholesterol suspended in 0.5 cc. of sesame oil. At intervals of 4 weeks the injections were repeated, so that a total of 200 mgm. of cholesterol was administered to each mouse in a period of just under 3 months. This material was well tolerated. Some mice showed persistent masses at the injection site.

Experiment 14. Cholesterol heated to 200° C.—Twenty mice were injected each with 50 mgm. of this cholesterol on each of four occasions, the conditions being exactly as in experiment 13. This material was well tolerated.

Experiment 15. Cholesterol heated to 300° C.—

Thirty mice were injected each with 200 mgm. of this cholesterol divided into 4 doses exactly as in experiment 13.

Carcinogenicity of Acrolein

Experiment 16.—Acrolein was made up so that 10 mgm. was contained in 0.5 cc. of sesame oil. When 10 mice were injected each with this amount they quickly became excited, went into shock, and died within 1 hour. When the amount of acrolein contained in 0.5 cc. of sesame oil was reduced to 1 mgm. and this was injected into each of 2 mice they both died within 7 hours. Consequently the dose adopted was 0.2 mgm. of acrolein dissolved in 0.1 cc. of sesame oil. This amount was injected into each of 15 mice at weekly intervals for 24 weeks. The total amount for each mouse was 4.8 mgm. of acrolein and 2.4 cc. of sesame oil.

Carcinogenicity of Heated Sesame Oil

Experiment 17.—Because of its wide use as a vehicle for many biological substances sesame oil was selected as an example of a vegetable oil for heating. It was slowly heated to 350° C. in an open vessel, and then allowed to cool. It began to darken and to fume vigorously at about 320° C.

This oil was injected into 31 mice. Each mouse was given 0.5 cc., followed at intervals of 4 weeks by 2 additional injections. Thus each mouse had a total of 1.5 cc. The material was well tolerated.

Three sarcomas occurred at the site of injection. Two were in males and one in a female. The first was discovered in the 12th month and the animal died in the 13th month with a tumor $35 \times 30 \times 18$ mm. which had ulcerated through the skin and infiltrated the muscles beneath. It was in direct contact with a mass of oil. Microscopic examination showed it to be a spindle cell sarcoma. There were no metastases.

The second sarcoma, discovered in the 20th month, killed its bearer before the month was out. It was 20 mm. in greatest diameter and had infiltrated both the overlying skin and the underlying muscle. Residual sesame oil was in contact with it. It, also, proved to be a spindle cell sarcoma; there were no metastases. (See Fig. 2.)

The third sarcoma was found in a female mouse, the last survivor in the experiment, that died on the 685th day after the first injection. The tumor, $15 \times 15 \times 5$ mm., was in direct contact with masses of injected oil. Microscopic examination showed this to be a spindle and mixed cell sarcoma.

Miscellaneous Tumors

In some of these experiments growths were encountered also in the mammary gland, the lungs, and the lymphatic system. The C57 brown mice had no such tumors, and the mice purchased on the open market had only a few, while the mice of our own stock had many more but well within the incidence previously reported (35). As there was no evidence that the incidence of these tumors was increased by the various substances fed or injected, the growths are considered spontaneous.

COMMENT

Four sarcomas were induced at the sites of injection of these various substances in experiments in which the total number of mice was 522, the number of 6 month survivors was 284, and the number surviving for over 12 months was 150, as is shown in the tables. In experiments 8 to 12 the mortality was unfortunately very high early in the course of the experiments. Taken alone, these experiments are not very significant, even though a few animals survived for periods of time which some have considered adequate. The results are included, however, because so far as they go they substantiate the results of the other experiments with meat extracts.

Though the experiments reported here are not exact duplicates of work reported by anyone else, they permit certain comparisons and correlations with respect to the sesame oil, the meats, and the cholesterol.

Sesame oil.—At 12 months the number of mice living that had been injected with unheated sesame oil, used as a vehicle for other substances in these experiments, was 61. None developed tumors at the site of injection. At the same time 9 mice were living that had been injected with sesame oil heated to 350° C., and of these, 3 developed sarcomas. Carcinogenic activity was therefore probably acquired in the heating of this oil. In other experiments (35, 33) unheated sesame oil likewise did not induce tumors in these mice. The heated sesame oil persisted longer at the site of injection than did the unheated oil, a factor that may be significant.

Schabad (31) reported that sunflower oil was not carcinogenic for mice, and Burrows, Hieger, and Kennaway (12) found this to be true for olive oil, linseed oil, and wheat germ oil. Beck (3), on the other hand, found cottonseed oil carcinogenic after heating to 340–360° C. for 1 hour.

When the evidence that heated vegetable oils are carcinogenic for rats is added to that obtained for mice it becomes evident that an exhaustive study of the carcinogenicity of various heated vegetable oils is imperative.

Meats.—The case for the carcinogenicity of the animal fats is not so convincing. One mouse injected with fat extracted with benzene from a mixture of overheated beef and pork died with a sarcoma at the

point of injection after 424 days. This tumor occurred in 1 of 18 mice that lived for 12 months or longer. However, at this time an additional 55 mice which had received injections of similar fats, or the nonsaponifiable fractions thereof, were living, as were 42 that had been fed these fats, yet none developed sarcomas. It is possible, of course, that the active substance is not present in the nonsaponifiable fraction, and that this was not the best method of concentrating the active principle.

Barry and Cook (2), Andervont (1), Burrows, Hieger, and Kennaway (12), and others, have injected animal fat into mice with negative results, and the group last named also injected lard which had been heated at 340–360° C. without inducing tumors, although the number of mice used was small.

TABLE III: COMMERCIAL MANUFACTURE OF LARD IN THE UNITED STATES *

Method	Prepared by this method, per cent	Maximum temperature reached, ° C.
Steam-rendered	80	150
Open-kettle-rendered	5	120
Dry-rendered	15	130

* Data supplied by Dr. Frank C. Vibrans, Chief Research Chemist, American Meat Institute, Chicago.

Even though injection experiments in mice have been uniformly negative in the hands of others, it is possible that the one tumor reported here may have been induced. The temperature was probably higher than that used in the commercial preparation of lard, which is said not to exceed 150° C. (26), but natural conditions in preparing meats for food were simulated, although slightly exaggerated.

The maximum temperatures used in the preparation of meats for human consumption are usually about 150° C., and are said rarely to exceed 200° C. This temperature, also, is seldom exceeded in the use of animal fats for deep frying (24), as in the preparation of doughnuts and potato chips, but here the fats are heated at this temperature repeatedly and for long periods of time. It is possible that harmful chemical reactions might occur gradually under these conditions which, at higher temperatures, might take place quickly.

Under these circumstances this subject merits further study despite the small amount of positive evidence presented here.

Cholesterol.—The situation with respect to cholesterol is less clear. The experiments of Kennaway and Sampson (18), who heated cholesterol to 800–810° C., thus forming a tar which was carcinogenic, are not comparable with those reported here. Neither are the experiments of Roffo (28, 29) because he fed rats, and their stomachs appear to be highly sensitive to many foodstuffs. The observation of Widmark (40), who induced one sarcoma in a mouse painted with cholesterol treated at 275° C., is difficult to evaluate in the absence of further information. It appears, then, that a final decision should be postponed until more work has been done with cholesterol.

SUMMARY

Numerous extracts made from overfried and overbaked meats were tested for carcinogenicity in mice by subcutaneous injection and by feeding.

1. The fats from a mixture of beef, pork, and suet that had been overfried were tested by injection, by feeding, and by injection of the nonsaponifiable lipids.

2. The nonsaponifiable lipids from a second mixture of beef, pork, and suet prepared from the raw meat, after overfrying and after overbaking, were tested by injection.

3. The nonsaponifiable lipids from overfried beef were tested by injection.

4. The nonsaponifiable lipids from a mixture of beef, pork, and lamb were tested by injection.

Only one sarcoma was induced at the site of injection of these meat extracts. This occurred in a mouse injected with the benzene-soluble fats from overfried mixed meats.

Pure cholesterol heated to 200° C. and to 300° C. was not carcinogenic on subcutaneous injection into mice. Acrolein also was not carcinogenic. Sesame oil that had been heated to 350° C. was carcinogenic, inducing 3 sarcomas in 9 mice that lived for more than one year.

These results, taken with those of others, indicate that further study of the carcinogenic potency of heated vegetable fats appears to be warranted.

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Diet and Epithelial Hyperplasia in the Forestomach of Rats and Mice

George R. Sharpless, Sc.D.

(From the Department of Laboratories, Henry Ford Hospital, Detroit, Mich.)

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Since Singer (19) in 1913 observed hyperplasia and ulceration of the forestomach epithelium in rats fed bread and wood shavings, there have been numerous studies to determine the etiology of the lesions. Pappenheimer and Larimore (14) showed that a faulty diet would produce the changes, but there is still divergence of opinion as to the nature of the deficiency and the mechanism of lesion formation.

While benign changes have been described by most authors, Fibiger (6) reported that squamous carcinoma of the forestomach epithelium was produced in rats and mice by feeding cockroaches infected with *Gongylonema neoplasticum* (*Spiroptera neoplastica*). He believed the evidence was conclusive that *Spiroptera* could serve as the etiologic agent of both benign and malignant changes. Carcinoma was thought to be a more advanced stage of the benign hyperplasia. Studies performed since the discovery of accessory food factors have shown that Fibiger's diet was nutritionally poor and that even without the worms it would induce some alterations (15). With an improved diet, Passey, Leese, and Knox (15) were able to increase the life span of infected animals without the production of extensive changes. Cramer (4) accepted Fibiger's interpretation of carcinoma in a few cases but made the reservation that something more than *Spiroptera* and poor diet was necessary to produce it.

Hoelzel and Da Costa (11) found ulcers in the epithelium of both the forestomach and the glandular portion in rats and mice when low protein diets were fed and also when the animals were fasted every second day. Since gastric stasis resulted from either method, they suggested that irritation from unbuffered acid produced the changes. Some support for this hypothesis was provided when Matzner and his associates (13) demonstrated that pepsin and hydrochloric acid could induce ulceropapillomas. These investigators observed that more extensive lesions developed when pepsin and hydrochloric acid were fed together than when either was given alone.

Irritation either by parasites or gastric juice is the only explanation that has been advanced for the mechanism of lesion formation, though without attempting to elucidate this mechanism numerous reports have suggested that the changes are a result of vitamin deficiency. Thus stomach lesions were found in rats suffering from vitamin B_1 deficiency by Dalldorf and Kellogg (5) and Sure and Thatcher (20), while Findlay (7) obtained them only with vitamin B_2 deficiency. These studies were made, however before the synthetic vitamins became available and other deficiencies also may have been present. Howes and Vivier (12) demonstrated that lesions occurred despite adequate protein intake if there was a vitamin B deficiency, and concluded that the whole vitamin B complex is necessary to maintain normal forestomach epithelium. Although Wolbach and Howe (22) proved that vitamin A is essential for the maintenance of normal epithelium in general, they did not find forestomach changes in vitamin A deficient animals. On the other hand, extensive lesions at this site were described by Fujimaki (9), and attributed to vitamin A deficiency, but further study (10) showed that undetermined factors were also important in their production. From a recent report of well controlled studies started by Fibiger (8) we learn that vitamin A deficiency under favorable conditions will increase the incidence of lesions, but that additional unknown factors are necessary completely to prevent their formation. While they offer no explanation of the mechanism of formation, Brunschwig and Rasmussen (1) suggest that when a certain degree of malnutrition exists in the rat, such malnutrition not necessarily being synonymous with absolute loss of weight nor with the lack of any specific type of food or vitamin, the forestomach mucosa reacts by localized foci of cellular proliferation.

Hyperplasia and ulceration are the normal reactions of the forestomach epithelium to mechanical or chemical irritation. Bullock and Rohdenburg (2) inserted into the forestomach by gastrotomy either a celluloid ball covered with pin points or pieces of cork with protruding pig bristles, and observed extensive changes, similar in many respects to those obtained by Fibiger with parasites. Chemical irritation with a piece of rubber sponge impregnated with Scharlach R, or pine tar oil introduced in a similar manner,

also stimulated epithelial hyperplasia in this region. Irritation from ingested hair was thought to be an etiologic agent in the bread flour regime of Pappenheimer and Larimore (14). However, the inclusion of 10 per cent chopped hair in a sufficient diet rarely produced changes, and although hair is normally ingested by rats in small amounts, forestomach changes are not found when an adequate diet is allowed. If hair is an etiologic factor it must be assumed, therefore, that the epithelium has been made abnormally sensitive by an incomplete diet.

By using two types of diet with rats of a single colony, Sharpless (17, 18) demonstrated that the lesions can be caused by more than one deficiency. A deficiency of protein in one regimen and a deficiency of the vitamin B complex in the other produced the changes. Adequate controls made it possible to correct the deficiency and get approximately normal stomachs without changing the other nutritive properties of the diet, and without affecting the growth of the rat. Recently Chen (3) found that agar-agar and glucose provided considerable protection in starved rats and questioned the theory that nutritional deficiency is important in the etiology of the lesions.

In all these studies, the lesions are believed to have been due directly to a dietary deficiency, to the action of local factors alone, or to local factors acting on epithelium with lowered resistance to irritation. The local factors that have been proposed are mechanical irritants such as hair, parasites, and hard particles in the food, as well as chemical irritants such as pepsin and hydrochloric acid. Bile that may have been regurgitated into the stomach is a second possible chemical irritant, although it has not been discussed in the literature in connection with changes in the rat's stomach. The divergence of reported results would probably be not great, if all the facts were known. However, some of them may be susceptible of interpretations different from those suggested by the authors. Using the two diets (17, 18) previously described, we have performed additional experiments to obtain data for an explanation of the lesions.

METHODS

In all experiments young rats, males and females between 55 and 80 gm. in weight, were used, and litter mate controls were fed the basal ration. Two types of diet were studied. One, the white flour diet, was composed of approximately 90 per cent white flour; the other, the low protein diet, was made up of purified foods low in protein. The experimental period was 42 days for the former and 90 to 100 days for the latter. Food and water were given ad libitum.

At the end of the experimental period all animals were killed with chloroform and their stomachs examined immediately. If one or more typical changes were found in the gross examination, the animal was recorded as having lesions. Except where the variation was great, no attempt was made to determine the protective power of the diet by differences in severity of the alterations.

RESULTS

The changes in the forestomach epithelium consist of well circumscribed ulceropapillomas that are usually round or oval in shape, although in some animals they blend and cover one-half or more of the surface. Sometimes the ulcerated portion is a narrow, irregularly elongated fissure, 5 mm. or more in length, surrounded by the typical thick epithelium. The border between the forestomach and the glandular portion is a slightly elevated ridge of squamous epithelium, called the limiting ridge. It is usually smooth, but in these animals it is more prominent and thickened irregularly in the form of beads. It appears to be one of the first areas affected, and may be prominent and beaded when no macroscopic ulceropapillomas are present.

The effectiveness of protective factors was determined in this study by the macroscopic appearance of the forestomach epithelium when the animal was killed. The changes were recorded as slight or mild when one to five small individual ulceropapillomas or beading of the limiting ridge were present. Severe or extensive changes were recorded when one-half or more of the surface was covered with ulceropapillomas.

Although sections were made of representative stomachs in all the groups studied, a detailed account of the microscopic alterations has not been included because they were essentially the same as those that have already been described so frequently.

Experiment 1.—The low protein diet previously described (17) had the following percentage composition: casein 4.0, dried yeast 5.0, salt mixture 4.0, butterfat 5.0, cornstarch 82. When 0.2 per cent cystine was added the stomach remained normal and growth was increased from 2 or 3 gm. to approximately 15 gm. per week. Protection under these conditions could have been provided either by cystine or by alleviation of chronic starvation. In order to eliminate the variation in intake, the food of 6 rats fed the cystine-supplemented diet was limited to that eaten by litter mate controls of the same weight fed the basal regimen. Two of the 6 fed cystine had one small lesion each, whereas the forestomach of each control was completely covered with lesions, a demonstration that sulfur-containing amino acid deficiency, and not starvation, was the main etiologic factor in the low protein diet.

Experiment 2.—Preliminary experiments showed that 12 per cent casein in the low protein diet described above is the minimum amount that will prevent the lesions. In order to test the theory that pepsin and hydrochloric acid will produce the change, 5.0 per cent scale pepsin was fed together with 12 per cent casein, and 0.05 N hydrochloric acid was given for drinking water. This diet was used in order to provide protection with as nearly as possible the same ration and conditions that produce the changes spontaneously. Of 9 rats given this diet 5 had one or more areas of typical ulceropapillomas, whereas their controls had normal stomachs, but in none of the 5 were the lesions so extensive as those caused by the low protein diet alone. This experiment demonstrated that although pepsin and hydrochloric acid could bring about changes, they alone were not responsible for the extensive lesions following the low protein regime.

Experiment 3.—A summary of some long continued feeding studies with the low protein diet is included under this experiment. Of 168 rats thus fed for more than 10 months, 3 have developed a squamous carcinoma in the mouth and one in the vagina. More than 1,800 animals of the same colony have had the normal stock ration for a similar period and no squamous carcinoma has occurred. Of 24 mice (C57 black strain) given the same diet for more than a year, 2 developed epithelial cysts on the tongue similar to those found in the stomach. This summary shows that with the low protein diet changes occur occasionally in areas of squamous epithelium other than the forestomach. The direct cause of these changes was probably some factor other than the diet, but in view of the experience with stock animals the regimen must have predisposed the rats to the action of this unknown factor.

Experiment 4.—The white flour diet had the following percentage composition: white flour 90.0, butterfat 5.0, calcium lactate 2.9, sodium chloride 2.0, and ferric citrate 0.1. The defect is a biologically poor protein and a deficiency of the vitamin B complex. The purpose of this study was to determine if a correction of the protein defect would prevent the changes. Eight rats were given the diet supplemented with 15 per cent casein, an amount known completely to prevent changes when the vitamins are adequate. Three of the 8 had stomach changes. Other experiments in which gelatin was added, or in which the supplements were pure amino acids, demonstrated conclusively that biologically poor protein was not the only cause of the lesions in rats fed white flour.

Experiment 5.—Previous studies have shown that practically normal forestomach epithelium can be maintained in the white flour diet with supplements of riboflavin, cystine, nicotinic acid, and a water-soluble concentrate of rice polishings (18). With pyridoxin and thiamin substituted for the rice polish concentrate, 31 of 49 rats had lesions. When choline was also fed only 8 of 58 animals had stomach changes. However, all 6 rats fed the white flour diet supplemented with choline alone had extensive stomach changes. Approximately 90 per cent of the animals fed the unsupplemented ration had abnormal forestomach epithelium. In none of these experiments did the supplements significantly improve the food consumption or growth.

Experiment 6.—In the studies mentioned above—except in the animals fed choline—intestinal contents were often found in the stomach. Choline caused a slight diarrhea and probably helped to prevent regurgitation of intestinal contents into the stomach. Since it is well known that bile is irritating, 0.5 per cent sodium taurocholate was incorporated in the supplemented white flour regime that gave the most complete protection. All 14 rats fed this diet had extensive changes in the forestomach epithelium while only 3 of 9 litter mate controls were affected, and each of these 3 had but one small abnormal area. In order to determine whether this was due to the action of sodium taurocholate alone or to the combined action of a poor diet and the bile salt, 9 rats were fed the normal stock ration supplemented by 0.5 per cent sodium taurocholate. Despite the fact that these animals ate 40 per cent more food, and therefore more sodium taurocholate, than those fed white flour, all had normal stomachs. Since the concentration of sodium taurocholate in contact with the stomach epithelium was probably similar in the two experiments, the stock diet must have made the epithelium less sensitive to irritants. As a whole these experiments show that in those diets where it may be regurgitated into the stomach, bile should be considered one of the etiologic agents of the stomach lesions only if the resistance of the epithelium has been impaired by a poor regimen.

Experiment 7.—In order to demonstrate further that the lesions in rats fed the white flour diet were not caused by chronic starvation, the food intake of 10 rats fed the normal stock ration was regulated so that their weight was kept constant. When examined at the end of the 6 weeks, the stomachs of all were normal. Of 9 litter mates fed the unsupplemented white flour diet, 8 had stomach changes. This leads to the conclusion that chronic starvation of the type that occurs with these deficient regimens does not allow sufficient contact between the epithelium and the local factors to produce the lesions.

DISCUSSION

If the lesions are the direct result of a dietary deficiency, areas of squamous epithelium in other parts

of the body should also present hyperplasia and ulceration, but despite the presence of extensive changes in the forestomach the squamous epithelium of the renal pelvis, vagina, and mouth are usually normal. Furthermore, if stomach lesions are produced by a specific inadequacy, this should always have the same effect. Some authors have referred stomach changes to a lack of protein, vitamin A, B_1, or B_2, yet with the same diets others have obtained no changes. On the basis of these observations it must be concluded that the lesions are not produced directly by incomplete regime.

Bullock and Rohdenburg (2) demonstrated that hyperplasia is the normal reaction of the forestomach epithelium to either mechanical or chemical irritation. However, it has been shown that hair, pepsin and hydrochloric acid, and bile, the irritants that can come in contact with the forestomach epithelium, are not capable of inducing epithelial changes when a good diet is fed. Experiment 6 indicates that a bile salt, sodium taurocholate, effected changes when the diet was poor. In experiment 2, pepsin and hydrochloric acid induced some changes in the stomachs of rats fed a suboptimum regimen. Together with reports in the literature on the effect of ingested hair, this evidence suggests that an incomplete diet makes the epithelium sensitive to irritants that normally do not affect it. That the forestomach mucosa may not have normal resistance to proliferative stimulation may also be deduced from the observation that changes occur occasionally in other areas of squamous epithelium.

In the long continued studies described in experiment 3, there were 4 cases of squamous carcinoma in areas other than the forestomach. Two mice developed epithelial cysts on the tongue that were similar to those found in the forestomach. Pyridoxin, riboflavin, and nicotinic acid are all known to be essential for the maintenance of normal squamous epithelium. Although in the 6 week experimental period dermatitis was not obtained in their absence, it must be assumed that a subnormal amount of the vitamins was present and that the epithelium had abnormal resistance to irritants.

One possible explanation of the mechanism for the formation of lesions was provided by the work of Hammett (see Reimann, 16). Hammett's theory is that $-SH$ is the essential stimulus for cell proliferation and that the strength of the required stimulus depends upon the amount of inhibitor (oxidized sulfhydryl) present. Furthermore, Reimann believes that either physical or chemical irritation will free $-SH$ locally. (If the concentration of the inhibitor were reduced, less irritation should be required for proliferation.) A deficiency of metabolizable sulfur such as that provided by the low protein diet, should give the necessary conditions for producing proliferation with less than normal stimulus. Although unassailable proof of this explanation must await further study, some similar chain of reactions is indicated by the experimental results.

In addition to lowering the resistance of epithelium to irritation, dietary deficiencies also provide an opportunity for prolonged contact between the local chemical irritants and the epithelium. Thus absolute starvation allows constant contact between the epithelium and the gastric juice. Subnormal insulation of the mucosa from local factors, and dilution of the gastric juice, would also be expected in partial starvation. Some bile probably enters the stomach during complete starvation because there is no material present to stimulate normal intestinal movement or to act as a physical barrier. Ingestion of some food or inert material should erect a physical barrier to this abnormal movement of bile unless the muscle tone of the intestinal tract were impaired or abnormal peristalsis occurred. Deficiencies of thiamin, riboflavin, and possibly some other members of the B complex impair muscle tone. Choline increases muscle tone of the intestinal tract (21). In the white flour diet alone it had no effect but with other members of the B complex it was an important factor in the prevention of lesions (experiment 6).

Regardless of nutritive value, it is clear from the above discussion that any substance that will either prevent bile from getting into the stomach or will insulate the epithelium from contact with the local factors should help to prevent changes in the forestomach. Agar-agar would serve as a physical obstruction to the regurgitation of bile, stimulate normal intestinal movement, and dilute as well as help to eliminate hydrochloric acid and pepsin. Chen's (3) observation of the protective action of glucose and agar-agar might be explained on this basis.

As a whole the experiments show that a large number of dietary constituents are concerned in maintaining normal conditions in the gastrointestinal tract. The action of these constituents is interdependent, so that a deficiency of one will prevent effective action by the others. In the dietary treatment of gastrointestinal derangements, therefore, better average results should be expected from therapy with the whole vitamin B complex together with biologically good protein than would be possible from large doses of one substance alone.

SUMMARY

Under the conditions described a deficiency of cystine, riboflavin, pyridoxin, or choline induced hyperplasia and ulceration of the forestomach epithelium in rats. Sodium taurocholate or pepsin and hydrochloric

acid increased the incidence of lesions in animals fed the poor diets, but caused no macroscopic changes in rats fed the stock ration. Neither did the limitation of stock diet intake cause any visible lesions in the forestomach, and the body weight remained constant in young rats.

The action of the protective factors is interdependent, so that a deficiency of one may prevent effective action by others. From these facts and the observation that squamous epithelial changes were occasionally observed in areas other than the stomach, it is postulated that the mechanism of formation of the gastric lesions is the irritation of abnormally sensitive epithelium by hair, hard food particles, pepsin and hydrochloric acid, or bile.

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Irradiated Benzpyrene and Inhibition of Urease Activity*

G. C. Mueller and H. P. Rusch, M.D.

(From the McArdle Memorial Laboratory, University of Wisconsin Medical School, Madison, Wis.)

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One approach to the question of how chemicals induce tumors is to search for evidence that carcinogens modify specific biochemical reactions. Since the carcinogenic hydrocarbons are insoluble in aqueous media, previous investigations from this laboratory have been confined to *in vitro* systems containing components such as phospholipids, fats, or oils in which the chemicals were soluble (6, 7, 17, 21). The recent observation of Brock and his associates (5) that aqueous solutions of these hydrocarbons can readily be prepared in the presence of purines such as xanthine, caffeine, or theobromine has greatly increased the number of enzyme systems which can be studied *in vitro* in contact with the carcinogens.

The relationship of cystine to growth and carcinogenesis has been investigated by various workers (16, 22) and suggested a study of the effect of benzpyrene on enzymatically functional sulfhydryl groups. Urease appeared to provide an excellent material for this purpose because considerable information exists about this enzyme and because the activity of urease is correlated with the integrity of its SH groups (10). Thus urease may be considered representative of certain other cellular enzymes. Preliminary experiments indicated that freshly prepared solutions of benzpyrene in aqueous caffeine did not affect urease activity, but that an increasing toxicity appeared after these solutions had been standing for several days in the light of the laboratory. The apparent influence of light on this carcinogen led to an investigation of the effect of irradiated benzpyrene on urease. Boyland had previously demonstrated in tissue metabolism studies (3, 4) that irradiated hydrocarbons were toxic.

MATERIALS AND METHODS

Arlco jack bean urease [1] was used in all experiments. Since this enzyme catalyzes the decomposition of urea,

$$H_2N-CO-NH_2+H_2O \longrightarrow 2NH_3+CO_2,$$

the reaction rate was followed in a Warburg apparatus by measuring the evolution of carbon dioxide in an acetate buffer pH 5.0 under the experimental conditions previously determined (15). All experiments were conducted in duplicate, and the compounds being tested were placed in contact with the enzyme for 15 to 20 minutes before the addition of the substrate. The final fluid volume per flask was 3 ml. in every case; a typical mixture consisting of 0.4 ml. M/2 urea in 1 M acetate buffer pH 5.0, 0.2 ml. Arlco urease (200γ dry weight), 0.9 ml. 1 M acetate buffer pH 5.0, and 1.5 ml. of H_2O or special reagent. The experiment was continued for 50 minutes, and only those variations in the final Q_{CO_2} which differed by more than 15 per cent from the control values were considered significant.

Benzpyrene [2] was dissolved in a small volume of concentrated aqueous caffeine solution with the aid of gentle heating and diluted with distilled water to a final caffeine concentration of 3 per cent. By this method, it was possible to prepare blue fluorescent solutions containing 150γ of benzpyrene per ml., although in the present study 3 per cent caffeine solutions containing 100γ benzpyrene per ml. proved to be most satisfactory. For irradiation, the solutions were placed in beakers, covered with an ultraviolet transmitting glass No. 791, and rayed with the light of an intermediate pressure mercury arc lamp at a distance of 20 cm. The periods of irradiation were varied as specified for each experiment. Upon exposure to light, the solution of benzpyrene readily changed to a golden brown color with a diminution of the fluorescence. A major portion of the brown material could be extracted in the following manner: The irradiated solution was thoroughly extracted several times with purified CCl_4, and this extract was washed with water 5 times to remove all traces of caffeine. Most of the solvent was then removed by distillation and the remainder evaporated in a vacuum desiccator, leaving a golden brown residue.

For the determination of hydrogen peroxide in the solutions two methods were employed: an iodometric titration (12) or an enzymatic decomposition by catalase. In the former method, 3 ml. aliquots of the

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[1] Sold by Arlington Chemical Company.

[2] Hoffmann-LaRoche.

H_2O_2-containing solutions were titrated with N/100 sodium thiosulfate after the addition of solid potassium iodide and 3 drops of 3 per cent ammonium molybdate. Under the experimental conditions, the starch indicator was sensitive to 2×10^{-5} molar H_2O_2. When hydrogen peroxide was decomposed by catalase, the volume of oxygen liberated was measured in a Warburg apparatus at 38° C. Crystalline catalase was prepared according to the method of Sumner and Dounce (20) and was redissolved in M/30 phosphate buffer at pH 7.4.

RESULTS

Freshly prepared solutions of benzpyrene in aqueous caffeine had little or no effect on the activity of the urease system, even when the benzpyrene concentration was raised to 320γ per ml. in 5 per cent caffeine solution. However, when solutions of benzpyrene in caffeine (100γ benzpyrene per ml. in 3 per cent caffeine), which had been irradiated for 1 hour, were added to the urease system, an 85 to 90 per cent inhibition of activity was observed. Irradiated caffeine alone reduced the urease activity by only 32 per cent (Table I).

TABLE I: INHIBITION OF UREASE ACTIVITY BY IRRADIATED SOLUTIONS OF BENZPYRENE IN AQUEOUS CAFFEINE

(100γ of benzpyrene per ml. in 3 per cent caffeine—control solutions of 3 per cent caffeine)

Substances tested	Irradiation time, minutes	Drying following irradiation	Inhibition, per cent
Caffeine control	0	—	0
Benzpyrene	0	—	0
Caffeine control	60	—	32
Caffeine control	360	—	56
Benzpyrene	60	—	85
Benzpyrene	360	—	93
Caffeine control	0	+	0
Caffeine control	60	+	0
Caffeine control	360	+	0
Benzpyrene	0	+	0
Benzpyrene	60	+	0-15
Benzpyrene	360	+	30
Caffeine (CCl_4 extract)	360	—	0
Benzpyrene (CCl_4 extract)	360	—	21
Hydrogen peroxide 3.4×10^{-4} M	0	—	88
Hydrogen peroxide 2.26×10^{-4} M	0	—	85
Hydrogen peroxide 1.16×10^{-4} M	0	—	71
Hydrogen peroxide 2.26×10^{-5} M	0	—	27

In an attempt to identify the toxic factors produced during irradiation, the solutions irradiated for one hour were dried in an oven at 100° C., redissolved volumetrically in distilled water, and then tested in the urease system. This procedure removed all the inhibiting effect from the irradiated caffeine solutions, and nearly all but a variable trace of the inhibitor from the radiated solution of benzpyrene in caffeine (Table I). Thus it appeared that the major inhibiting factor was either volatile or unstable to the drying process.

When the period of irradiation was increased from 1 hour to 6 hours, the toxicity of the caffeine control was also abolished by drying, but a 30 per cent inhibition persisted with the corresponding benzpyrene-caffeine solution (Table I). These results indicated that at least two types of toxic factors were produced by the irradiation of benzpyrene-caffeine solutions, one of which disappeared on drying and the other of which was heat-stable and was detected only after prolonged irradiation. Since the stable factor appeared to originate from the hydrocarbon, attempts were made to extract the product with organic solvents. Extraction with carbon tetrachloride was found to remove most of the color from the irradiated solution, and tests made with these extracts on urease indicated that the colored portion of the solution contained the inhibitor (Table I). This toxic derivative was probably similar to those noted by Boyland, since prolonged irradiation was necessary for adequate demonstration in both experiments (3, 4).

TABLE II: EFFECT OF CATALASE ON THE TOXICITY OF IRRADIATED SOLUTIONS OF BENZPYRENE IN AQUEOUS CAFFEINE

(100γ of benzpyrene per ml. in 3 per cent caffeine—control solutions of 3 per cent caffeine)

(Irradiated one hour)

Substance tested	Q_{CO_2}	Inhibition, per cent	Iodine test for H_2O_2
Caffeine control	1,690	0	0
Irradiated caffeine	1,255	26	Slight
Irradiated benzpyrene	255	85	Strong
Caffeine control + catalase.	1,835	0	0
Irradiated caffeine + catalase	1,815	1	0
Irradiated benzpyrene + catalase	1,605	14	0

Since the toxic factor, which was stable to the drying process and extractible with carbon tetrachloride, accounted for only a small part of the total inhibition, the identification of the volatile inhibitory substances became of interest. The presence of a peroxide or some similar agent was suggested by the fact that potassium iodide was oxidized to I_2 as demonstrated with the iodine-starch test. The iodine tests became negative when catalase was added to the irradiated solutions (Table II). Since catalase rapidly decomposes only hydrogen peroxide, the presence of the latter material was indicated (19).

The concentration of hydrogen peroxide in the irradiated solutions was determined manometrically by

measuring the decomposition of the peroxide by catalase. Calculations based on these data were limited to solutions containing not less than 10^{-4}M H_2O_2 and agreed closely with the results obtained by titration with thiosulfate. In order to demonstrate an increased production of hydrogen peroxide in the presence of benzpyrene, solutions were irradiated and the hydrogen peroxide concentrations determined after 1, 2, 4, and 6 hour periods. During the early stages of irradiation approximately 6 times as much hydrogen peroxide was produced in the presence of benzpyrene as when caffeine alone was irradiated; and this ratio, 6:1, increased to 9:1 at the end of 360 minutes (Table III). Apparently benzpyrene functioned as a photosensitizing catalyst in the formation of H_2O_2.

TABLE III: THE RATE OF H_2O_2 PRODUCTION IN IRRADIATED SOLUTIONS OF BENZPYRENE IN AQUEOUS CAFFEINE

(100γ of benzpyrene per ml. in 3 per cent caffeine—control solutions of 3 per cent caffeine)

(H_2O_2 determinations by thiosulfate titration)

Length of raying period, minutes	Hydrogen peroxide molarity: Irradiated caffeine	Hydrogen peroxide molarity: Irradiated benzpyrene-caffeine	H_2O_2 ratio Caffeine: benzpyrene
60	2.0×10^{-5} M	1.2×10^{-4} M	1:6.0
120	3.0×10^{-5} M	1.9×10^{-4} M	1:6.3
240	4.0×10^{-5} M	2.7×10^{-4} M	1:6.7
360	6.0×10^{-5} M	5.4×10^{-4} M	1:9.0

When solutions of hydrogen peroxide were added to the urease system in approximately the same concentrations as those found in the irradiated solution of benzpyrene, the degree of inhibition observed was essentially equal to that obtained with the irradiation products themselves (Tables I and III). Furthermore, when catalase was used to decompose the hydrogen peroxide in the irradiated solutions of caffeine prior to their addition to the urease system, no inhibition of urease activity was observed; and the addition of catalase to irradiated solutions of benzpyrene in caffeine removed all but 14 per cent of the original inhibition. This small remaining inhibition probably resulted from the presence of substances derived from the hydrocarbon (Table II).

DISCUSSION

The results of this experiment indicate that at least two types of substances inhibitory to urease activity are formed by the irradiation of solutions of benzpyrene in caffeine: one, a product soluble in organic solvents and probably derived from benzyprene, and the second, hydrogen peroxide. The production of hydrogen peroxide in water during irradiation with ultraviolet light has been previously demonstrated (9), and our results suggest that benzpyrene acts as a photosensitizing catalyst in this reaction. A parallel observation has been reported by Blum and Spealman (1) who showed that H_2O_2 was formed in irradiated aqueous solutions of fluorescent dyes.

A number of investigators have observed that some hydrocarbons have a photolethal effect on various types of living cells when such organisms are exposed to the light, whereas no toxic influence is detected if light is excluded (8, 11, 13, 23). The catalytic effect of benzpyrene on the formation of H_2O_2 suggests a possible mechanism for the photolethal action of these hydrocarbons. It appears quite probable that the presence of the hydrocarbon in the cell might catalyze the formation of intracellular peroxide when light is applied. Doniach has observed the ingestion of hydrocarbon particles by paramecia (8), and these organisms remained light-sensitive even after the hydrocarbon had been removed from the medium. Information on the concentration of catalase in the organisms used in these studies would be of interest since low amounts of catalase would enhance the probability of death due to the hydrogen peroxide produced. Liebert and Kaper (14) found that the lethal factor for bacteria, which is produced when bacterial media containing erythrosin and methylene blue are irradiated, is hydrogen peroxide, and that the lethal activity is abolished by brushing the surface of the medium with a catalase solution.

At the present time, however, it seems doubtful whether the photodynamic action of the hydrocarbons plays a role in the mechanism of carcinogenesis. Light does not appear to be necessary in the production of tumors by hydrocarbons, for tumors can readily be produced in parts of the body that light does not reach. Furthermore, there is no conclusive evidence available that light supplements the action of the hydrocarbons when both are applied to the skin, and ultraviolet irradiation and carcinogenic hydrocarbons do not appear to act synergistically in the production of skin tumors in mice (18). Consequently, if the hydrocarbon plays the role of an intermediate in the conversion or transformation of energy to some vital process of carcinogenesis, it must utilize some source of energy other than light. Although there are no experimental data to substantiate this suggestion, it might be possible for hydrogen peroxide to furnish energy for such a process. Blum has shown that hemolysis is produced in the dark by lower concentrations of dye when hydrogen peroxide is present (2).

SUMMARY

Benzpyrene was dissolved in aqueous solutions of caffeine and its effect on the activity of a urease system determined. Freshly prepared solutions were relatively nontoxic to the enzyme, but irradiation of the

hydrocarbon solution with ultraviolet light produced at least two types of substances which definitely inhibited urease activity. One of these agents could be detected only after prolonged irradiation and presumably was derived from the benzpyrene, while the second inhibitory substance proved to be hydrogen peroxide. Apparently benzpyrene acted as a photosensitizing catalyst in the production of H_2O_2 during ultraviolet irradiation.

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The Effect of *p*-Dimethylaminoazobenzene on the Formation of Blood Proteins*

B. E. Kline, M.S.**

(From the McArdle Memorial Laboratory, University of Wisconsin Medical School, Madison, Wis.)

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The mechanism of tumor production by chemical carcinogens might be clarified considerably if definite changes in a body constituent could be demonstrated during the precancerous period, as distinguished from those during the cancerous state itself. The carcinogenic azo dyes show a high degree of specificity in producing tumors of the liver and hence might be expected to affect certain hepatic functions.

Of the various liver functions with which the azo dyes might interfere the most interesting is protein synthesis, because of the probability that carcinogenesis involves some slight, but permanent, alteration in protein metabolism (8). It is possible that such a change would lower the level of one or more of the blood proteins which are thought to be formed in the liver (albumin, fibrinogen, or hemoglobin). If so, the effect could be quickly and easily detected since these proteins can be accurately determined in small samples of blood.

That protein synthesis can be altered experimentally has been demonstrated repeatedly. Liver poisons, such as chloroform and carbon tetrachloride, cause a lowering of albumin and fibrinogen, and high protein diets tend to alleviate this condition (1, 5, 9, 12). Similarly, the feeding of *p*-dimethylaminoazobenzene to rats causes a reduction in hemoglobin (15).

In the present study, *p*-dimethylaminoazobenzene was fed to rats, and blood samples were withdrawn at intervals during the precancerous period for the determination of fibrinogen, albumin, and hemoglobin. For comparison, similar determinations were made on the blood of rats that had large liver tumors but that had not recently received any azo dye.

METHODS

Sprague-Dawley albino rats, 175 to 250 gm. in weight and under 6 months of age, were divided into 4 groups of 10 each. They were bedded on shavings and were fed as follows:—

Group 1.—Basal diet (controls). Cerelose,[1] 80; crude casein, 12; salts, 4 (14); corn oil, 2; cod liver oil, 1; and vitab,[2] 1.

Group 2.—Basal diet + 0.06 per cent *p*-dimethylaminoazobenzene dissolved in the corn oil. The animals in this group were allowed food ad libitum and ate an average of 8.5 gm. per day.

Group 3.—Same as group 2 + 0.01 per cent *p*-aminobenzoic acid added to the vitab. This supplement was added because anthranilic acid has been reported to decrease the toxicity of the azo dye (10).

Group 4.—Same as group 2 + 50 μgm. riboflavin[3] per rat per day. This was added to improve the general condition of the animals since the vitab in the diet supplied less than 1 μgm. per rat per day.

The animals in groups 1, 3, and 4 were restricted to 9 to 10 gm. of food per day so that each consumed the same amount during the whole experiment.

In addition, 7 rats with large liver tumors (group 5) were maintained on a dog biscuit diet. These animals were from a previous experiment (11); they had received no azo dye for at least 60 days prior to analysis.

The effects of a reduced food intake caused by the toxicity of the dye and the fast before the taking of blood samples were determined by analyzing the blood of stock rats for blood proteins before and after a starvation period of 4 days.

Blood samples.—After 18 hours of fasting the rats were anesthetized with ether and the area over the heart was moistened with 70 per cent alcohol; 2 to 2.5 cc. of blood were withdrawn from the heart into a 2 cc. syringe which had been rinsed with heparin (1:1,000 in Ringer's solution). The blood was oxalated, centrifuged at 3,000 r.p.m. for 20 minutes, and the plasma drawn off. Occasional samples clotted; in these, serum albumin and total nitrogen values were determined.

Fibrinogen.—Of a 12.5 per cent Na_2SO_3 solution, 9.5 cc. were added to 0.5 cc. plasma in a graduated

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** Finney-Howell Research Fellow.

[1] A pure commercial glucose monohydrate.

[2] "Vitab rice bran concentrate" was generously furnished by Dr. A. E. Briod, of National Oil Products Company, Harrison, N. J.

[3] Dr. D. F. Robertson, of Merck and Company, kindly furnished this vitamin.

test tube, and the mixture was heated at 37° C. for 10 minutes (2). The coagulated fibrinogen was centrifuged, washed with 12.5 per cent Na_2SO_3, and recentrifuged. The washed protein was digested as described below.

Albumin.—Of a 21 per cent Na_2SO_3 solution, 9.5 cc. were added to 0.5 cc. plasma (or serum) and the mixture was filtered after it had stood for 30 minutes (2). One cubic centimeter of the filtrate was taken for digestion as described below.

RESULTS AND DISCUSSION

The rats in group 1 (control) remained in good condition throughout the experiment with no loss in weight. One of them died of hemorrhage following heart puncture after 5 months. Of the 10 rats receiving the azo dye (group 2), one died at 2 months, one died and one was killed at 3 months, and the rest were killed at 4 months because of their emaciated condition. At 4 months, 2 had liver cancer (one with metastasis to the lung), 3 showed pronounced cirrhoses with malignant changes, and 2 livers were negative. When *p*-aminobenzoic acid was fed with the dye (group 3), 2 rats died at 2 months (one of them from hemorrhage following heart puncture); one died at 4 months from hemorrhage; one died at 5 months; and one was killed at 5 months with liver adenocarcinoma, hepatoma type II (4), and metastases to the lungs. The 5 survivors were killed at 6 months—4 with liver cancer and 1 negative. There was very little cirrhosis in this group. The effect of adding *p*-aminobenzoic acid to this diet was striking; food consumption was always the maximum allowed, the animals remained in good condition, survival (except for accidental deaths) was excellent, the incidence of cancer was high, and gross liver cirrhosis was prevented. The addition of riboflavin to the diet (group 4) improved the condition of the animals to such an extent that none of them died. They were killed at 6 months at which time there was no liver cancer and only slight cirrhosis. Thus the addition of a protec-

TABLE I: THE EFFECT OF *p*-DIMETHYLAMINOAZOBENZENE ON CERTAIN BLOOD PROTEINS

(Values expressed as gm. per 100 cc., averages, and ranges)

		Groups				
	Months	1 Basal diet	2 Azo dye	3 Dye + *p*-aminobenzoic acid	4 Dye + riboflavin	5 Liver tumors
Fibrinogen	2	0.38(0.22–0.73)	0.48(0.32–0.80)	0.26(0.20–0.35)	0.37(0.24–0.48)	
	3	0.30(0.21–0.40)	0.49(0.43–0.56)	0.26(0.21–0.30)	0.33(0.28–0.39)	
	4	0.30(0.24–0.47)	0.41(0.21–0.55)	0.31(0.27–0.38)	0.33(0.27–0.39)	
	5	0.39(0.27–0.52)		0.36(0.26–0.47)	0.35(0.25–0.50)	
	6	0.30(0.24–0.46)		0.31(0.24–0.38)	0.38(0.30–0.59)	0.55(0.39–0.87)
Albumin	2	4.3(3.1–4.5)	3.3(2.9–3.8)	3.1(2.6–4.5)	4.0(3.7–4.6)	
	3	4.1(2.0–5.0)	3.9(2.8–4.4)	3.8(3.4–4.4)	3.8(3.6–4.0)	
	4	4.0(3.1–4.4)	3.4(2.2–4.4)	3.7(3.6–4.0)	4.1(3.9–4.4)	
	5	4.2(3.5–4.6)		3.8(3.3–4.1)	4.0(3.2–4.4)	
	6	4.2(3.7–5.0)		4.3(4.1–4.4)	3.7(3.1–4.2)	4.3(3.6–5.1)
Total nitrogen	2	1.25(1.0–1.5)	0.98(0.8–1.2)	1.20(0.9–1.7)	1.10(0.98–1.2)	
	3	1.30(1.2–1.5)	1.10(0.9–1.3)	1.10(0.9–1.2)	1.05(1.0–1.1)	
	4	1.25(1.1–1.3)	1.2(1.1–1.4)	1.26(1.0–1.4)	1.20(1.1–1.3)	
	5	1.10(1.0–1.2)		1.24(1.1–1.4)	1.13(1.1–1.2)	
	6	1.25(1.1–1.5)		1.15(1.1–1.2)	1.13(1.0–1.2)	1.24(1.0–1.5)
Hemoglobin	6	14.7(12.7–16.9)		11.5(10.5–12.6)	11.4(9.5–12.6)	13.3(10.9–15.2)

Total nitrogen.—A 0.05 cc. sample of plasma (or serum) was digested as follows:—

Digestion.—The samples prepared for digestion (7) were placed in pyrex test tubes graduated at 10 cc. Two cubic centimeters of 10 N H_2SO_4, containing 1 gm. $CuSeO_3$ per liter, were added, and the water was removed in a drying oven at 110–120° C. The tubes were then placed in a wire basket and heated at the high heat of a hot plate until the solution was clear and colorless. A small glass bulb placed in the top of each tube helped condense the vapor and prevent loss of sample.

The digested solution was cooled and diluted with water to 10 cc. One cubic centimeter of this solution was treated with 2 cc. of water, 2 cc. of Nessler's solution containing gum ghatti, and 3 cc. of 2 N NaOH (7). The method was modified [4] slightly at this point by the use of a Cenco spectrophotolometer to measure, at 520λ, the intensity of color developed.

[4] Dr. V. R. Potter. Unpublished data.

tive factor to the diet obviated the effects of possible "side reactions" of the carcinogenic agent.

No definite changes in the blood proteins appeared either as the result of the azo dye (groups 2, 3, and 4, Table I), of the presence of liver tumors (group 5, Table I), or of starvation (Table II). Variations within the groups were usually larger than variations between group averages. The single exception appeared to be in the level of hemoglobin in which the controls (group 1) averaged 14.7 gm. per 100 cc. of blood as compared to 11.5 and 11.4 gm. for animals which had received the dye for 6 months (groups 3 and 4). This confirms a previous observation (15). The effect on hemoglobin appeared to be due to the toxicity of the dye, *i. e.* a "side reaction," since it was evident in both group 3 with liver cancer and group 4 with no cancer, and since the rats with large liver tumors (group 5) had a nearly normal hemoglobin figure (13.3 gm. per 100 cc. blood).

TABLE II: EFFECT OF STARVATION ON PLASMA PROTEINS

(Values expressed as gm. per 100 cc., averages, and ranges)

	Stock diet	4 day fast	Over-all average Group 1
Fibrinogen	0.41(0.37–0.44)	0.25(0.17–0.31)	0.33
Albumin	4.3(4.0–4.7)	3.9(3.3–4.5)	4.2
Total nitrogen	1.34(1.2–1.4)	1.20(1.1–1.3)	1.2

The level of fibrinogen was reduced by fasting (Table II), but not by the presence of the azo dye. The relative constancy of the levels of albumin and fibrinogen in the presence of the azo dye, of cirrhosis, or of liver tumors confirms the well known fact that certain functions of the liver proceed normally in spite of extensive damage to that organ.

The hope of finding a chemical change characteristic of the pre-cancerous condition has not been realized. Others have had similar experiences, for Orr and Stickland (13) reported that glycolysis in liver tissue does not change until a tumor is formed; DuBois and Potter (3) did not find a reduction in liver cytochrome *c* until the liver tumors were well advanced; and Greenstein and his associates (6) saw a reduction in liver catalase only when the animal had a liver tumor or tumor transplant.

Perhaps we should not expect to find a precancerous change, since the number of cells that are being changed into cancer cells at any one time is probably very small in proportion to the total number of liver cells. Thus the carcinogen might cause a pronounced change in the metabolism of these few cells without producing any measurable difference in the metabolism of the total liver.

SUMMARY

Liver cancer was produced in rats by feeding purified diets containing *p*-dimethylaminoazobenzene. Plasma albumin, fibrinogen, and total nitrogen were measured at intervals during the carcinogenic period, and hemoglobin was determined at 6 months. There were no changes in blood proteins which could be associated with the process of carcinogenesis. When the diet contained *p*-aminobenzoic acid, cirrhosis was greatly reduced without changing the incidence of liver cancer.

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SECTION A

GROWTH OF MAMMARY TUMORS AND NODULES IN HYPOPHYSECTOMIZED MICE. W. U. GARDNER. (Yale University School of Medicine, New Haven, Conn.)

Published in *Cancer Research*, **2**:476-488, 1942; abstract p. 581.

OBSERVATIONS ON THE ADRENALS OF CASTRATE MICE AND THE RELATION OF THESE ADRENALS TO CANCER. GEORGE W. WOOLLEY, ELIZABETH FEKETE, and C. C. LITTLE. (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.)

In order to lay a basis for further study of strain variation as well as to learn more about the early stages of nodular hyperplasia and to study sex differences, a series of castrate and control males and females of the JAX dba strain were killed in early life at monthly intervals. Changes noted at two and succeeding months of life are described. A similar study with the JAX ce strain shows changes leading to carcinoma of the adrenal cortex. A masculinizing effect of the carcinoma is noted.

A COMPARISON OF THE GROWTH OF DEER ANTLER AND OF OSTEOGENIC SARCOMA. JOSEPH C. AUB and GEORGE WISLOCKI (by invitation). (Collis P. Huntington Memorial Hospital of Harvard University, and the Harvard Medical School, Boston, Mass.)

The growth of deer antlers is faster than that of osteogenic sarcoma, but it is beautifully organized and regulated. Growth of the antlers starts while the testes are still atrophic, and it is therefore assumed that the initiating impulse comes from elsewhere, probably the pituitary. The testes, however, are involved in the maturation and shedding of the antlers. Chemical analyses of blood and spinal fluid throughout the year indicate that the parathyroid glands are not overactive during antler growth. Chemical studies on the antler indicate that it behaves like membranous bone with some addition of a horn-like substance. Antlers and osteogenic sarcoma have the same intracellular enzyme, phosphatase, which obviously arises from the osteoblasts. As the antlers mature, the phosphatase content of the tissue decreases to the level found in normal adult bone. This is in sharp contrast to the findings in osteogenic sarcoma. Another interesting difference between the two is that the enormous production of phosphatase in the antlers is not obvious in the blood level.

THE ESTRADIOL CAPACITY OF THE LIVER IN MICE OF CANCER SUSCEPTIBLE AND CANCER RESISTANT STRAINS. G. H. TWOMBLY and H. C. TAYLOR, JR. (Memorial Hospital, New York, N. Y.)

Published in *Cancer Research*, **2**:811-817. 1942; abstract p. 852.

THE PRESENT STATUS OF THE METABOLISM OF THE ESTROGENS. R. I. DORFMAN and W. R. FISH. (Western Reserve University, School of Medicine, Cleveland, Ohio.)

Read by title; abstract not available.

THE LIVER CATALASE ACTIVITY OF NORMAL AND OF TUMOR-BEARING RATS AND MICE. JESSE P. GREENSTEIN, JULIUS WHITE, and HOWARD B. ANDERVONT. (National Cancer Institute, Bethesda, Md.)

The liver catalase activity of animals bearing a wide variety of tumors was found to be between one-tenth and one-twentieth the activity of the normal liver. At the present time, two general statements may be made concerning this phenomenon: (a) the effect on the liver catalase is progressive with the growth of the tumor, the fast growing tumors producing the effect much earlier than the slow growing ones, and (b) the effect is reversible, for removal of the tumor, either by excision or by spontaneous regression in certain strains of mice, is followed by a rapid restoration of the liver catalase to the normal level. The only exceptions have been found in mice bearing extremely slow growing transplanted tumors, such as a hepatoma in C3H mice and an intestinal adenocarcinoma in A mice, and in rats bearing the Jensen sarcoma in which the growth of the tumor has been unaccountably slowed.

THE ENZYME SPECTRUM IN NORMAL AND CANCEROUS TISSUES OF HEPATIC AND OF MAMMARY ORIGIN. JESSE P. GREENSTEIN. (National Cancer Institute, Bethesda, Md.)

Every tissue is characterized by a number of enzyme systems of specific activity. The description of a tissue in

terms of the activity of a number of its component enzymes provides a method of distinguishing the tissue from others, just as inspection of the emission or absorption spectra in the case of simple molecules serves as a means of identification of the molecules. When this method of using the enzyme spectrum to identify tissues is applied to pairs of homologous tissues, such as tumors and their respective tissues of origin, it also provides the possibility of following the changes from the normal to the cancerous condition.

The activity of the following enzymes was determined in normal liver and in transplanted hepatic tumors in rats and mice, as well as in hyperplastic mammary tissue and in the spontaneous mammary tumors of mice: arginase, catalase, xanthine dehydrogenase, amylase, thymonucleodepolymerase, acid and alkaline phosphatase. The results showed that each tissue can be described in terms of the activity of its component enzymes. Comparison of the tumors with homologous normal tissues revealed that certain enzymes have the same activity in both, whereas others are considerably different. The distribution of the enzymes in certain cases varied with the species used as well as with the different strains of mice. It is clear that the activity of one or two enzymes cannot be used to characterize a single tissue or a pair of homologous normal and tumor tissues. The composite picture of the whole spectrum of several enzyme systems is indispensable in any such characterization.

STUDIES ON THE PATHOGENESIS OF INDUCED LEUKEMIA. D. P. McENDY and J. FURTH. (Cornell University Medical College, New York, N. Y.)

In a study of the pathogenesis of induced leukemia the disease was produced in mice by repeated percutaneous applications of methylcholanthrene. The animals were killed at various intervals, blood-forming organs examined microscopically, and transmission experiments made with suspensions of spleen and lymph nodes. In the preleukemic state there were in the spleen and lymph nodes large lymphoid follicles with large germinal centers containing an increased number of mitotic figures. Since transmission experiments were negative, these changes are not likely to be neoplastic. The earliest three cases of leukemia were first demonstrated by transmission experiments. Subsequent microscopic review showed the presence of collections of atypical cells in the splenic pulp and in lymph nodes, while the bone marrow and liver showed no change. Thus, of the tests used to determine the presence of leukemia, gross examination is the least reliable, microscopic examination next in order, and inoculation the most decisive. The type of leukemia produced was, with few exceptions, lymphoid or atypical. Evidence is presented indicating that these atypical cells originate in lymphoid tissues. The onset of induced leukemia is sudden when judged by the criteria studied.

METABOLISM OF MOUSE TISSUES IN THE PRELEUKEMIC STATE. DEAN BURK, HERBERT SPRINCE (by invitation), MARY F. BOON (by invitation), and JACOB FURTH. (National Cancer Institute, Bethesda, Md., and Cornell University Medical College, New York, N. Y.)

Three to 6 week old mice were painted twice a week for 18 weeks with 3 per cent methylcholanthrene in benzene. Some of the animals were also irradiated with 300 r. The anaerobic fermentation, aerobic fermentation, and respiration of the lymph nodes, spleens, and livers of treated animals were compared before and after the onset of leukemia with those of litter mate untreated controls, and the observations correlated with histologic and transmission studies. The anaerobic fermentation (lactic acid formation) was the metabolic property of chief interest, since, although by no means of itself specific for malignant tumor metabolism it is, so far as known, an invariable characteristic thereof.

No differences in metabolism between control (unpainted) and preleukemic tissues were found, although statistically significant differences of less than 20 to 30 per cent are not precluded by this statement. There was considerable variation in the metabolic values, over the order of a twofold range. The anaerobic fermentation values ($Q_A^{N_2}$=cu. mm. lactic or other acid formed per hour per mgm. initial dry weight for spleen ranged from 4 to 7, for lymph node from 3 to 8, and for liver from 0.4 to 0.9, with a tendency to increase with the age of the animal in the case of spleen and lymph node, and probably to decrease in the case of liver.

The anaerobic fermentation values of spleen and lymph node of leukemic animals usually showed no change during early stages after onset, but values during advanced stages often rose to 10 to 12, which was also true when nonleukemic leukopoiesis developed. With liver, however, $Q_A^{N_2}$ increased noticeably immediately and simultaneously with the histopathologic indication of the onset of disease and the correlated successful transmission; values from 1 to 10 developed, depending upon the degree of infiltration. The liver metabolism provides, therefore, an excellent indication of leukemogenesis that is characteristic although not specific.

Section B

HETEROLOGOUS TRANSPLANTATION OF A HUMAN FIBROSARCOMA. H. S. N. GREENE. (Yale University School of Medicine, New Haven, Conn.)

Published in *Cancer Research*, **2**:649-654; abstract p. 736.

BLOOD EOSINOPHIL FACTOR IN THE RESISTANCE OF THE RABBIT TO THE BROWN-PEARCE TUMOR. ALBERT E. CASEY. (Department of Pathology and Bacteriology, School of Medicine, Louisiana State University, New Orleans, La.)

The pretransplantation level of the blood eosinophils was compared (1) with the success of transplantation of the neoplasm, (2) with the incidence and number of metastases, and with the longevity and mortality among animals successfully transplanted. Of 195 apparently healthy male rabbits of various breeds and hybrids, 87 (45 per cent) were discarded because one or more of nine blood factor levels were abnormal (*i.e.*, mean 1.96). The 108 animals remaining were inoculated intratesticularly

with the Brown-Pearce tumor. The pretransplantation blood eosinophil level was highly related to the development of metastases and to the mortality from the tumor among the 90 animals successfully inoculated. There was evidence that the eosinophil was in some way related to the immune reaction of the rabbit to the Brown-Pearce tumor. High pretransplantation blood eosinophil levels were related to fewer metastases, to a regression of the transplanted tumor, and to a lower mortality in animals successfully inoculated. Low pretransplantation eosinophils were associated with the contrary. No relation of the pretransplantation eosinophil level to the success of transplantation was found. The blood eosinophil factor perhaps represented a strong secondary line of defense against the neoplasm.

NATURE OF THE BACTERIAL POLYSACCHARIDE WHICH CAUSES HEMORRHAGE IN MOUSE SARCOMA. J. L. HARTWELL (by invitation), M. J. SHEAR, and J. R. ADAMS, JR. (by invitation). (National Cancer Institute, Bethesda, Md.)

Further data were given on the material (Hartwell, Shear, Kahler, and Turner, *Cancer Research*, **1**:741. 1941) obtained from cultures of *B. prodigiosus* (Shear and Turner, *J. Biol. Chem.*, **133**:lxxxvii. 1940), which causes hemorrhage in transplanted and primary subcutaneous tumors in mice (Shear, *Cancer Research*, **1**:731-732. 1941).

Although the polysaccharide fraction obtained previously had given negative tests for protein, it reacted positively to colorimetric tests for certain amino acids—notably tyrosine, arginine, and histidine. Tryptic digestion reduced the nitrogen and acetyl content to half without detectable loss of potency. Removal of the residual protein-like material did not change the nondialyzable nature of the active agent.

Treatment for 12 hours with 0.1 *N* NaOH at room temperature or with 2 *N* HCl at 37°, destroyed the potency. The action of NaOH was accompanied by loss of the entire acetyl content. Treatment with 0.1 *N* $NaHCO_3$ at 37° removed most of the acetyl without loss of potency.

The polysaccharide did not reduce Fehling's solution. Maximum hydrolysis was obtained with 1.5 *N* HCl at 100° for 2½ hours when about 60 per cent of reducing sugars, calculated as glucose, was found. About 16 per cent of a water-insoluble, alkali-soluble product containing nitrogen and phosphorus was also obtained. The hydrolysate formed a crystalline osazone resembling glucosazone. Amino sugar constituted about 4 per cent of the reducing sugars when calculated as glucosamine. Methyl pentose accounted for about 30 per cent of the reducing sugars. Hydrolysis was accompanied by a change in specific optical rotation from +128° to +36°. Negative results were obtained in tests for pentoses, keto sugars, desoxy sugars, and uronic acids. Individual hexoses shown to be absent were galactose, mannose, and inositol.

The current bioassay technic was described. The purest preparations contained about 10,000 mouse tumor units per mgm.; the end point dose, *i.e.* that which regularly caused hemorrhage in 50 per cent of the tumors, was about 0.1 microgram.

ANAPHYLAXIS AND HEMORRHAGE INTO TUMORS. MORRIS K. BARRETT. (National Cancer Institute, Bethesda, Md.)

Published in *J. Nat. Cancer Inst.*, **2**:625-630. 1942; to be abstracted in *Cancer Research*.

THE EFFECT OF INJECTION AND INGESTION OF AMINO ACIDS UPON THE APPEARANCE, GROWTH, AND REGRESSION OF THE PERRY SARCOMA IN RATS. HOWARD H. BEARD. (Louisiana State University School of Medicine, New Orleans, La.)

For the last 10 years studies have been conducted with adrenal extracts which contained proteoses and peptones which proved of some value in causing both experimental and clinical malignant tumors to disappear. The present report covers the effect of administration of the amino acids isolated from this extract upon the Perry sarcoma in rats.

The amino acids were secured from Merck & Company, Rahway, N. J., and were dissolved in dilute NaOH, followed by neutralization with dilute HCl to pH 7.1 with a Beckman pH meter. In 3 liters of solution there were dissolved the following amounts in grams of the amino acids: *d*-arginine-HCl, 15; *d*-histidine-HCl, 3.6; *d*-glutamic acid, 15; *dl*-phenylalanine, 6; *dl*-alanine, 18; *l*-leucine, 6; *dl*-valine, 14; *l*-cystine, 15; *l*-proline, 3; *l*-hydroxyproline, 3. The whole was then made up to 6 liters with sterile distilled water, preserved with merthiolate, and kept in the ice chest.

One or 2 cc. of this mixture were injected daily except Sunday around the tumor, depending on its size (5 or 10 gm.). In another group approximately 30 times this amount was ingested daily by the rats in their drinking water. Injection of the mixture caused the complete disappearance of 38 out of 46 tumors, and drinking the amino acid mixture caused 83 out of 120 tumors to disappear. In this group 8 tumors again began to grow very slowly, and 10 did not grow more than 5 or 10 gm. during the last 2 months.

In a group of older rats bearing larger tumors the injection of the mixture served to prolong life, caused a few tumors, one of 100 gm., to disappear completely, and held the growth of others stationary.

At the present time each amino acid is being tried alone for its effect on the Perry sarcoma.

FURTHER DATA ON THE OCCURRENCE IN WHOLE BLOOD OF MATERIAL INFLUENCING THE INCIDENCE OF MAMMARY CARCINOMA IN MICE. GEORGE W. WOOLLEY, L. W. LAW, and C. C. LITTLE. (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.)

This is a further report of an investigation of the possible role of blood in influencing the incidence of mammary carcinoma in mice. The recipient and control mice were of the second inbred generation following the nursing of high mammary tumor JAX C3H mice on JAX C57 black mice. At 1 to 3 months of age 2 females of each breeding pen of 4 females were injected with 0.5 cc. of whole blood diluted with an equal part of distilled water. Normal

males and females of the inbred C3H strain were used as donors. Significant differences in tumor incidence now appear between the injected mice and their litter mate controls. The increase in mammary carcinoma incidence was obtained following inoculations of whole blood from (1) males, (2) young females, and (3) lactating females.

DATA ON WHICH REPORT IS BASED

Mice	Number of animals	Number of tumors	Percentage of tumors	Average age at appearance of tumor, days	Number dying tumor-free	Average age dying tumor-free, days
Experimental	121	33	27.27	334.6	47	359.47
Control	119	10	8.40	308.1	43	294.76

18.87 ± 4.95
$D/ = 3.81$
$P = 0.00014$

THE RELATIONSHIP OF DIETARY DEFICIENCIES TO THE OCCURRENCE OF PAPILLARY ATROPHY OF THE TONGUE AND LEUKOPLAKIA OF THE ORAL MUCOUS MEMBRANES. J. C. ABELS, P. REKERS (by invitation), H. MARTIN, and C. P. RHOADS. (Memorial Hospital, New York, N. Y.)

Published in *Cancer Research,* **2**:381-393. 1942; abstract p. 524.

OCCURRENCE OF A TUMOR IN THE ADRENAL OF A CASTRATE RAT. W. C. HUEPER. (Warner Institute for Therapeutic Research, New York, N. Y.)

Published in *Cancer Research,* **2**:294-295. 1942; abstract p. 379.

AFTERNOON SESSION—MARCH 31, 1942

SECTION A

THE GENESIS OF EXPERIMENTAL SKIN CANCER BY METHYLCHOLANTHRENE. WILLIAM CRAMER and ROBERT E. STOWELL (by invitation). (Barnard Free Skin and Cancer Hospital, St. Louis, Mo.)

Published in *Cancer Research,* **1**:849-852. 1941; abstract p. 905; and in *J. Nat. Cancer Inst.,* **2**:369-378, 379-402. 1942; to be abstracted in *Cancer Research.*

THE INCIDENCE OF PRIMARY LUNG TUMORS IN MICE WITH INDUCED SARCOMAS. CHARLES E. DUNLAP and SHIELDS WARREN. (New England Deaconess Hospital, Boston, Mass.)

Published in *Cancer Research,* **2**:685-688; abstract p. 800.

THE EFFECTS OF BREATHING TOBACCO SMOKE ON STRAIN A MICE. EGON LORENZ, HAROLD L. STEWART, JOHN H. DANIEL (by invitation), and CLIFFORD V. NELSON (by invitation). (National Cancer Institute, Bethesda, Md.)

An automatic tobacco smoking machine was used to expose mice for several hours daily to tobacco smoke. Ninety-seven experimental and 97 control mice (strain A) of both sexes were kept in a chamber through which the smoke passed, for from 25 to 250 days. The maximum time was 693 hours. The average smoke content of the air in the chamber was 1 mgm. per liter. Calculations gave the amount of tar deposited in the respiratory passages of any one mouse during the entire run as roughly 0.5 gm.

No lung tumors were induced by the tobacco tar, for the average number of spontaneous pulmonary tumors was the same in the experimental as in the control animals. Pigmented foci were observed in the lungs of all mice exposed for more than 3 months, probably indicating areas of deposited tar. A few experimental animals showed inflammatory lesions of the stomach and duodenum. Further studies are now in progress.

PRODUCTION OF SUBCUTANEOUS TUMORS IN MICE WITH AIR DUST TAR. JOSEPH LEITER (by invitation), and M. J. SHEAR. (National Cancer Institute, Bethesda, Md.)

Published in *J. Nat. Cancer Inst.,* **3**:155-165. 1942; to be abstracted in *Cancer Research.*

ADENOCARCINOMA OF THE GLANDULAR STOMACH IN MICE INJECTED WITH 20-METHYLCHOLANTHRENE. HAROLD L. STEWART and EGON LORENZ. (National Cancer Institute, Bethesda, Md.)

The carcinogenic hydrocarbons 20-methylcholanthrene, 1,2-benzpyrene, and 1,2,5,6-dibenzanthracene were introduced directly into the wall of the glandular chamber of the stomach in mice of both sexes and from strains C3H, A, C, C57 black, I, and dilute brown. The dose of carcinogen varied from 0.2 to 0.8 mgm. Four vehicles were employed, as follows: a solution in mineral oil containing 6.8 mgm. of hydrocarbon per cc.; a solution in lard containing 0.4 mgm. of hydrocarbon per cc.; a dispersion in horse serum containing 20 mgm. of hydrocarbon per cc.; and cotton thread, impregnated with approximately 0.5 mgm. of the hydrocarbon and stitched directly into the wall of the glandular stomach. The solutions and dispersions were injected into the wall of the glandular stomach by means of a tuberculin syringe and a 27-gauge hypodermic needle.

In all 293 mice were used and at autopsy the following tumors were found: in the glandular stomach, adenoma 44, adenocarcinoma 8, adenoacanthoma 8, mixed adenocarcinoma and sarcoma 18, mixed adenoacanthoma and sarcoma 2, sarcoma 29; in the forestomach, squamous papilloma 44, and squamous carcinoma 14. Some animals had more than one tumor. Possible explanations for the development of tumors of the forestomach are (1) occasional escape of some of the injected material into the wall of the forestomach during injection, and (2) transport of carcinogen by peristaltic action or by lymphatics following injection.

In all the experiments except those with the impregnated cotton thread, some type of glandular tumor either in pure form or mixed with sarcoma or squamous elements was induced. In the experiment with the impreg-

nated cotton threads 19 mice were employed but only one tumor, a sarcoma, was observed at autopsy.

Adenocarcinoma of the glandular stomach was induced in 6 male C3H mice which received 0.8 mgm. of methylcholanthrene dispersed in horse serum and in 2 male I mice which received 0.6 mgm. of methylcholanthrene in the same vehicle and in addition 0.14 mgm. of methylcholanthrene in mineral oil. The adenocarcinomas in these 2 strain I mice arose at the site of injection of the horse serum dispersion of methylcholanthrene. The mice which developed gastric adenocarcinoma were 13 to 26 weeks of age when the injection was made. The interval between the time of injection and the finding of the gastric neoplasm at autopsy in these 8 mice was 19, 22, 23, 33, 37, 38, 39, and 46 weeks. In all 8 the tumor extended through the muscularis and infiltrated the peritoneum, but extragastric extension and metastases were not observed. One adenocarcinoma, transplanted subcutaneously into mice of the same strain, is now in the 8th generation and continues to reproduce the glandular characteristics of the original growth. This transplant reaches a diameter of 15 mm. in from 2 to 4 weeks.

With regard to the other malignant tumors, extragastric extension and metastases to the liver, pancreas, mesentery, or peritoneum occurred as follows: adenoacanthoma 1, sarcoma 9, mixed adenocarcinoma and sarcoma 6, mixed adenoacanthoma and sarcoma 1.

The location of the adenoacanthomas is interesting. Four involved both the glandular stomach and the forestomach so that their origin from both squamous and glandular mucosa is probable. The other four tumors were confined to the glandular stomach, and therefore the only possible site for their origin would be the glandular mucous membrane.

THE FAILURE OF METHYLCHOLANTHRENE TO ACCELERATE THE PAPILLOMA-TO-CANCER SEQUENCE IN VIRUS-INDUCED PAPILLOMAS ON COTTONTAIL RABBITS. JEROME T. SYVERTON, E. BUIST WELLS (by invitation), and GEORGE PACKER BERRY. (Department of Bacteriology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.)

Rous and Kidd have reported the prompt appearance of carcinomas when papilloma virus was injected intravenously into domestic rabbits whose skin had been "prepared" by tar. Since we have found that methylcholanthrene readily induces carcinomas and sarcomas in rabbits, it seemed desirable to study methylcholanthrene in this type of experiment. Accordingly, 1 per cent methylcholanthrene in benzene was applied to both ears of 27 cottontail rabbits, which we employed because the virus is readily recoverable from papillomas on this host. Each received 32 to 42 applications over a period of 150 to 220 days.

The rabbits fall into two groups. Group A comprises 15 animals that received methylcholanthrene only to test its carcinogenicity. When autopsied after 244 to 783 days following the first application, 12 had epidermoid carcinomas, 7 with metastases. Each of the 12 rabbits in group B received intravenously more than a million infective doses of papilloma virus between the 197th and 231st day after the first application of methylcholanthrene. After 10 to 20 days, typical papillomas appeared at the aural and at scarified cutaneous control sites. Their evolution was typical and virus was repeatedly obtained at successive biopsies. (The methylcholanthrene-induced papillomas, including those that progressed to overt malignancy, yielded no virus.) These 12 cottontails in group B died between the 233rd and 495th days. Eleven had malignant tumors, 4 with metastases.

We found no evidence, therefore, that methylcholanthrene accelerates the papilloma-to-carcinoma sequence in virus-induced papillomas on cottontail rabbits.

ATTEMPTS AT EARLY DIAGNOSIS OF CANCER OF THE CERVIX EXPERIMENTALLY INDUCED IN MICE. EDGAR ALLEN, W. U. GARDNER, and VINCENT J. COLLINS (by invitation). (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

A recent report that it is possible to diagnose early carcinoma of the uterus in women by a study of vaginal smears (Papanicolaou and Traut, 1941) seemed to promise a simple method free from possible complications of the trauma incident to cervical biopsy.

A number of female mice in our colony had been injected with estrogens frequently for long periods. A high percentage of those surviving for more than a year develop cervical lesions of varying degrees (Gardner, Allen, and Strong, 1936; Allen and Gardner, 1941). Vaginal smears were taken from 30 of these, beginning before the appearance of lesions, stained by the method described for diagnostic use in women, and read independently by three investigators. Some of the larger cervical lesions were diagnosed from the smears.

THE MORPHOLOGY AND DEVELOPMENT OF TESTICULAR TUMORS IN MICE OF THE A STRAIN RECEIVING ESTROGENS. C. W. HOOKER (by invitation) and C. A. PFEIFFER (by invitation). (Yale University School of Medicine, New Haven, Conn.)

Published in *Cancer Research*, **2**:759-769; abstract p. 726.

THE EFFECTS OF PREGNANT MARE SERUM UPON THE COURSE OF DEVELOPMENT OF TESTICULAR TUMORS IN MICE OF THE A STRAIN. C. A. PFEIFFER (by invitation) and C. W. HOOKER (by invitation). (Yale University School of Medicine, New Haven, Conn.)

Treatment with estrogens for 8 months or longer leads to the development of interstitial cell tumors of the testis in mice of the A strain. As the stages in the development of the tumors suggest that the effects of the estrogen might have been mediated by the hypophysis, pregnant mare serum (25 r.u. daily) was injected into 18 males of the A strain for periods of 3 to 20 months. The interstitial cells increased in number with the formation of discrete nodules, resembling stages in the development of tumors evoked by estrogen. The increase in the number of interstitial

cells appeared to be due to differentiation of new cells rather than to hyperplasia of preexisting Leydig cells.

When mice of the A strain were treated with estrogen to the point of early formation of new Leydig cells, and the pregnant mare serum was substituted for the estrogen, tumor formation proceeded. Mere cessation of the injections of estrogen at the same stage was followed by complete recovery of the testis.

Also to test other factors in the physiology induced by estrogen injections, testes from litter mates were transplanted into 12 female mice at birth. The interstitial cells of these grafts hypertrophied and after 12 to 16 months definite nodules, identical with an early stage of tumor development, were found.

BENIGN AND MALIGNANT MAMMARY LESIONS PRODUCED BY METHYLCHOLANTHRENE AND CRYSTALLINE ESTROGEN IN THE RAT. MILTON J. EISEN. (Department of Cancer Research, Columbia University, New York, N. Y.)

Published in *Cancer Research,* **2**:489-493. 1942; abstract p. 583.

THE DISTRIBUTION AND PREVALENCE OF A CARCINOGENIC FACTOR IN HUMAN LIVERS. PAUL E. STEINER. (Department of Pathology of the University of Chicago, Chicago, Ill.)

Published in *Cancer Research,* **2**:425-435. 1942; abstract p. 521.

Section B

THE ORIGIN OF SOME INBRED STRAINS OF MICE. L. C. STRONG. (Department of Anatomy, Yale University School of Medicine, New Haven, Conn.)

Published in *Cancer Research,* **2**:531-539. 1942; abstract p. 665.

STUDIES ON THE CONTROL OF MAMMARY CANCER INCIDENCE IN MICE. W. S. MURRAY. (Biological Station, Springville, N. Y.)

A report of experiments designed to illustrate the variation in concentration of the mammary cancer-stimulating substance transmitted through the mother's milk in mice.

OBSERVATIONS ON THE GENETICS OF SUSCEPTIBILITY TO THE DEVELOPMENT OF BREAST CANCER IN MICE. JOHN J. BITTNER. Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.)

Published in *Cancer Research,* **2**:540-545. 1942; abstract p. 664.

A GENETIC ANALYSIS OF SUSCEPTIBILITY TO INDUCED PULMONARY TUMORS IN MICE. W. E. HESTON. (National Cancer Institute, Bethesda, Md.)

Published in *J. Nat. Cancer Inst.,* **3**:69-78. 1942; to be abstracted in *Cancer Research.*

FURTHER OBSERVATIONS ON THE EFFECT OF FOSTER NURSING ON THE GROWTH OF TRANSPLANTABLE LEUKEMIAS IN MICE. L. W. LAW. (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.)

The resistance against progressive growth of inoculated leukemic cells characteristic of certain refractory strains of mice has been significantly reduced by foster nursing of refractory mice by mothers of the susceptible strain.

Three (possibly 4) leukemias, an induced lymphoid leukemia, line L1449; a spontaneous lymphosarcoma, line P1534, both in the dba strain of mice; and a myeloid leukemia, line C1498, in the C57 black strain of mice, grow progressively in some refractory strains or substrains that have received a "susceptibility influence" through foster nursing by females of the strain of origin of the leukemia tested. On the other hand 4 leukemias, myeloid and monocytic, a fibrosarcoma, 2 adenocarcinomas, a melanoma, and normal homologous tissues have not been affected in their growth in refractory mice through a "susceptibility influence."

The "susceptibility influence" is present throughout most of the normal lactation period. Total deprivation of susceptible milk in young mice of the susceptible strain does not affect their response to inoculations of leukemic cells. In the case of 2 leukemias tested, line L1449 and line P1534, the "susceptibility influence" was found to be transferred to second generation animals.

Attempts to transmit the influence to young mice by (1) inoculations of normal tissues, (2) normal tissue extracts given orally and parenterally, and (3) tissue filtrates and extracts have not been successful.

A change in the growth capacity of leukemic cells of 2 lymphoid leukemias and a myeloid leukemia whereby leukemic cells can be grown by serial transfer in refractory hosts, following initial growth in refractory foster nursed animals, has been observed.

REACTIONS OF HYBRIDS AND "PSEUDO-HYBRIDS" TO INOCULATIONS OF MOUSE TUMOR C198. ARTHUR M. CLOUDMAN. (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.)

Published in *Cancer Research,* **3**:47-52. 1943; abstract p. 142.

A COMPARISON OF BENIGN AND "MALIGNANT" LESIONS IN DROSOPHILA MELANOGASTER. ELIZABETH SHULL RUSSELL (by invitation). (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.)

Five benign tumor strains and a "malignant" one are described in *Drosophila melanogaster* as to time of appearance, activity, structure, location, and manner of inheritance, and the results of transplantation given.

All, including the so called "malignant" one, could be successfully transplanted without hampering the development of the host. Larvae of the lethal, tumor-producing type were all found to develop a gut abnormality causing midgut occlusion. Death occurred probably from starvation rather than from the effects of the tumors.

It is concluded that all tumors tested in *Drosophila,*

although not identical in inheritance, are very similar in structure and behavior, and that none is malignant. It is suggested that pupation may restrict these abnormal growths and prevent their becoming malignant.

METHODS FOR DETERMINING THE RELATIVE IMPORTANCE OF THE MAJOR CLASSES OF ENVIRONMENTAL FACTORS AFFECTING TUMOR FORMATION. W. LAWSON RUSSELL (by invitation). (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.)

Inbreeding has shown that variation in the environment is an important factor in tumor formation. Although isolated natural and experimental environmental influences have been studied by many investigators, little information has been obtained on the relative importance of the main classes of naturally occurring environmental factors. Thus, even in mammary cancer in mice, where an agent transmitted by the milk is likely to remain the most interesting single nongenetic factor, it can be shown that the remaining, and as yet unanalyzed, environmental influences play a greater role.

Methods for separating environmental factors into those common to sibs, those common to litter mates, and those which act differently on individuals within a single litter or even on opposite sides of the same individual, have been used by Wright, Reed, Green, and others in analyzing the variation of several characters in mammals. These methods have yielded unexpected and valuable results. In particular, they have uncovered the relative importance of environmental factors that might be controlled and those that are beyond control at the present time. An extensive application of these methods to various types of tumors in several inbred strains of laboratory mammals should furnish suggestions as to the relative efficacy of various ways of reducing the incidence of cancer in human populations.

THE RELATIVE INCIDENCE OF CANCER ACCORDING TO SITE, AGE, COLOR, AND SEX. C. C. LITTLE. (Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.)

Presents an analysis of United States census figures tabulated in a new way which shows the relative rather than the absolute incidence of cancer.

ON THE GEOGRAPHIC DISTRIBUTION OF GASTRIC CANCER IN THE STATES OF THE UNION. WILLIAM CRAMER. (Barnard Free Skin and Cancer Hospital, St. Louis, Mo.)

The work of Collins, Gover, and Dorn presented at the Gastric Cancer Conference organized in 1940 by the National Advisory Cancer Council has revealed great differences in the recorded incidence of gastric cancer in the various States of the Union. This communication is an attempt to analyze some of the factors responsible for these differences.

The recorded incidence of gastric cancer varies from a maximum of 31 per 100,000 population for Minnesota to a minimum of 11 per 100,000 population for Arkansas.

The stomach belongs to the group of sites for which the diagnosis of cancer is so difficult that it is apt to be missed, and the recorded, *i.e.* diagnosed, incidence of cancer thus tends to be less than the actual incidence at these sites. The extent of this error depends on the available diagnostic facilities, the measure of which is the number of physicians available for the population. This ratio varies from 473 persons to 1 physician in the State of New York to 1,532 persons to 1 physician in Kentucky. In all the states with a ratio of 900 persons or less to 1 physician the recorded incidence of gastric cancer is uniformly low, indicating an extensive under-diagnosis of gastric cancer. This error is likely to obscure for these states any differences in etiological factors which may exist.

In the states with better diagnostic facilities the recorded incidence, while generally higher, still shows considerable variations. While errors due to under-diagnosis may be partly responsible for some of those, the statistical data available from some European countries demonstrate the existence of extensive variations that are not due to inequalities in diagnostic facilities but indicate the existence of differences in etiological factors. Thus for some European countries a definite relationship between gastric cancer and social status has been established, gastric cancer being more frequent in the lower than in the higher social classes, suggesting the possibility that it is to a considerable extent a preventable disease.

These considerations indicate that if the etiological factors for gastric cancer are to be investigated by a comparison between different states, the inquiry should at first be limited to a small number of states so selected as to have similar diagnostic facilities with a different incidence of gastric cancer. An alternative study of differences among various occupations or social classes should not extend to all the states of the Union but should be limited to one or a few possessing optimal diagnostic facilities.

A QUANTITATIVE STUDY OF THE RATE OF GROWTH OF A TRANSPLANTABLE MAMMARY ADENOCARCINOMA OF THE MOUSE. J. G. HOFFMAN, H. GOLTZ, and S. WARNER. (State Institute for the Study of Malignant Diseases, Buffalo, N. Y.)

Experimental data on the volume growth of transplantable mammary tumor are correlated with the average cell volume growth and the latent period of the tumor. Cell suspensions, whose potency was measured by their cell count, were inoculated and the tumor volume measured as a function of time. The data show that the volume growth of two tumors, the E060 and the Marsh-Simpson tumor, is exponential with time having constants in the exponent of 0.3008 per day and 0.35 per day respectively. The exponential law of growth indicates that these tumors exist in the microscopic state for the greater part of their life span. The rate of volume growth of the individual cells is shown to be at least exponential.

SYMPOSIUM ON DIET IN RELATION TO CANCER

FOLLOWING THE ANNUAL DINNER, MARCH 31, 1942

1. JULES ABELS. Thiamin Metabolism in Patients with Leukemia.

2. C. A. BAUMANN. Effect of Diets High in Fat.

3. DEAN BURK, JULIET M. SPANGLER, and RICHARD J. WINZLER. Biotin-Avidin-Vitamin Balance in Pro-, Anti-, and Amphicarcinogenesis.

4. CHARLES KENSLER. Diet and *p*-Dimethylaminoazobenzene Cancer.

5. ALBERT TANNENBAUM. Effects of Calorie-Restricted Diets.

6. JULIUS WHITE. Dietary Sulfur.

MORNING SESSION—APRIL 1, 1942

SECTION A

COMPARISON OF X-RAY AND NEUTRON EFFECTS ON DIFFERENT STAGES OF MITOSIS. ALFRED MARSHAK. (Radiation Laboratory, University of California, Berkeley, Calif.)

Read by title; abstract not available.

RADIOSENSITIVITY OF MOUSE SARCOMA 180 IN TISSUE CULTURE. ROBERT CHAMBERS and C. G. GRAND (by invitation). (Washington Square College, New York University, New York, N. Y.)

Localized tumors, about 1 cm. in diameter and of 7 to 8 days' growth, were exposed *in vivo* to varying dosages of hard x-rays. They were then immediately removed, some fragments transplanted into normal mice and others planted in tissue culture. The tissue cultures were grown for varying lengths of time and then transplanted back into mice. The dosages used were 2,000 and 10,000 r, all of which are lethal for the tumor if it is left *in situ*.. Transplants of the tumors irradiated with 2,000 r grew successfully *in vivo* and *in vitro* and the explants, up to 28 days of culturing, produced successful growths when inoculated into mice. Transplants of tumors irradiated with 10,000 r also gave rise to growths *in vivo* and *in vitro*. However, the latter, when planted back into mice, produced tumors provided the cultures were implanted a short time only after culturing. These cultures showed progressive signs of deterioration, and the longer they were cultured the less able were they to succeed when transplanted back into mice.

These experiments indicate that 2,000 r produce no cumulative effect on the cells *per se* since the cells grow well if they are removed from the animal and transplanted after having grown in tissue culture for as long as 28 days. The deteriorative effects of the extraordinarily high dosage of 10,000 are exhibited only in the tissue cultures, since if the irradiated tumor is transplanted immediately into another mouse it grows successfully.

EFFECT OF X-RADIATION ON HYPOTONIC CYTOLYSIS OF SEA URCHIN EGGS. C. G. GRAND (by invitation) and ROBERT CHAMBERS. (Washington Square College, New York University, New York, N. Y.)

Irradiation of unfertilized sea urchin eggs (*Arbacia*) increases greatly the percentage of hypotonic cytolysis. A dosage of 2,500 r (200 kv., unfiltered) produced 40 per cent cytolysis within 10 minutes, while nonradiated controls showed a cytolysis of only 10 per cent; 5,000 r induced 50 per cent cytolysis; 10,000 r, 70 per cent cytolysis; and 20,000 r, 90 per cent cytolysis.

These results might be interpreted as indicating an increase in the osmotically active fraction, within the cell, with consequent increase in the rate of swelling followed by cytolysis.

However, it has been found that the procedure alone of weakening or removing the extraneous coats of the eggs (the vitelline membrane in the case of the *Arbacia* eggs) enhances the cytolytic effect of hypotonic solutions. It is also known that x-radiation weakens and tends to destroy the vitelline membrane. It is concluded, therefore, that the increased hypotonic cytolysis induced by x-radiation is to be referred to the effect of the irradiation on the extraneous coats. This conclusion is strengthened by the fact that irradiated fertilized eggs with fertilization membranes which are resistant to irradiation withstand hypotonic cytolysis to the same degree as nonradiated eggs.

THE PRODUCTION OF TUMOR-LIKE GROWTHS BY IRRADIATION OF SPERM. P. S. HENSHAW. (National Cancer Institute, Bethesda, Md.)

To be published in *J. Nat. Cancer Inst.*, **3**. 1943; to be abstracted in *Cancer Research*.

CELL VIABILITY AS A FACTOR IN INDUCING RESISTANCE TO MALIGNANT GROWTH. ANNA GOLDFEDER (by invitation). (Division of Cancer, Department of Hospitals, and the Department of Experimental Surgery, New York University Medical College, New York, N. Y.)

Fragments of a highly malignant tumor (sarcoma 180) irradiated with 2,200 r prior to implantation failed to produce any palpable tumor. The mice, however, remained resistant to subsequent nonirradiated tumor implants. This acquired resistance to subsequent tumor implants was demonstrable during a period of 8 days to 10 months following the first implantation of the irradiated tumor fragments.

Similar observations were made with mice that grew tumors from implants previously irradiated with smaller doses of x-rays (500 to 1,800 r) but these tumors eventually regressed. The acquired resistance to subsequent implants was not only local, but also generalized, since no tumor growth took place following implantation of tumor fragments at any site.

On the other hand, tumor implants that were previously irradiated with high doses of x-rays (60,000 r) and failed to produce any detectable tumor in the animal, also failed to induce any resistance to subsequent implantation of the same type of tumor. These animals developed tumors within 8 to 10 days following implantation of viable, nonirradiated tumor fragments. There was no difference in the latent period or the percentage of takes among animals previously implanted with tumor fragments irradiated with 60,000 r as compared to control animals, even when the subsequent nonirradiated implant was placed at the same site as the previous irradiated one.

The doses of irradiation mentioned above were arrived at from previous work (*Radiology*, 1940 and 1941), where it was demonstrated that whereas tumor fragments of

sarcoma 180 failed to produce any visible growth *in vitro* following irradiation with about 60,000 r, only 2,000 to 2,200 r were sufficient to prevent proliferation of the same type of tumor fragments implanted subcutaneously into animals.

Two main points may be brought out from these observations in regard to immunity.

1. Completely inactivated tumor cells (for instance, with 60,000 r for sarcoma 180) failed to induce any detectable resistance in animals to proliferation of subsequent tumor implants.

2. Resistance to transplantable tumors can be induced by living cells or by one of their constituents which is not destroyed in this case by comparatively small doses of x-rays.

The degree of resistance induced by irradiated tumor may indicate to a certain extent the degree to which its cells are devitalized by a given dose of radiation.

THE EFFECT OF *o*-AMINOAZOTOLUENE ON ULTRAVIOLET IRRADIATION. H. P. RUSCH and B. E. KLINE (by invitation). (McArdle Memorial Laboratory, Medical School, University of Wisconsin, Madison, Wis.)

Ultraviolet irradiation at levels of approximately 5.4×10^7 ergs per sq. cm. for 30 minutes daily was administered to albino mice for several months. Diets containing 0.05 per cent of *o*-aminoazotoluene were given to these mice during the period of irradiation or for varying periods before raying. The rate of formation of ultraviolet tumors was accelerated whether the diet was administered during or prior to irradiation. The time required for 50 per cent of the mice to develop them was about one month less in those receiving aminoazotoluene. The combination of ultraviolet irradiation and dye was rather toxic and caused considerable inflammatory redness and thickening of the ears, referable, probably, to photosensitization by the dye. Cancer formation was not confined to the ears but developed also on the top of the head in some of the mice receiving both carcinogens over the same period. The additive effect of aminoazotoluene and irradiation is especially interesting since similar results were not obtained with combinations of various other carcinogenic agents and ultraviolet irradiation.

KINETICS OF TUMOR INDUCTION IN MICE BY ULTRAVIOLET RADIATION. HAROLD F. BLUM, HUGH G. GRADY, and JOHN S. KIRBY-SMITH (by invitation). (National Cancer Institute, Bethesda, Md.)

Published in *J. Nat. Cancer Inst.*, **3**:91-97. 1942; to be abstracted in *Cancer Research*.

PENETRATION OF SKIN BY ULTRAVIOLET RADIATION AS A FACTOR IN CARCINOGENESIS. JOHN S. KIRBY-SMITH (by invitation), HAROLD F. BLUM, and HUGH G. GRADY. (National Cancer Institute, Bethesda, Md.)

Published in *J. Nat. Cancer Inst.*, **2**:403-412. 1942; to be abstracted in *Cancer Research*.

HISTOLOGIC TYPES OF CUTANEOUS TUMORS INDUCED BY ULTRAVIOLET RADIATION IN STRAIN A MICE. HUGH G. GRADY, HAROLD F. BLUM, and JOHN S. KIRBY-SMITH (by invitation). (National Cancer Institute, Bethesda, Md.)

Cutaneous tumors of the external ear induced in strain A mice by ultraviolet radiation according to a technic previously described are predominantly fibrosarcomas, derived from the subepidermal connective tissue. Smaller numbers of carcinomas, arising from the epidermis, are observed. The preponderance of tumors originating beneath the epidermis is consistent with the fact that tumor-inducing wave lengths penetrate to these tissues. That such radiation is capable of inducing tumors derived from cells other than the fibroblast or epidermal cell is evidenced by the fact that this series includes such histologic types as hemangioendothelioma, osteochondrosarcoma, and sebaceous carcinoma.

THE EFFECT OF X-RAYS UPON VIRUS-INDUCED PAPILLOMAS ON COTTONTAIL RABBITS. J. T. SYVERTON, S. L. WARREN, H. E. DASCOMB (by invitation), and G. P. BERRY. (Departments of Bacteriology and Radiology, University of Rochester School of Medicine and Dentistry, Rochester, N. Y.)

Our investigations of the effects of x-rays upon papilloma virus have shown that in cell-free suspensions it is highly resistant to roentgen radiation (millions of r units are required for inactivation), while cutaneous papillomas induced by it on domestic rabbits are easily eradicated by 3,500 or more r units. These findings are supported and explained by the present study on cottontail rabbits, used because the virus is usually recoverable from its papillomas though rarely recoverable from papillomas on domestic rabbits.

Massive doses of from 3,700 to 8,000 r units (23 cm. target distance to papilloma base; 90 kv.; no filter) so damaged the tissues and tumors that both frequently sloughed. Nevertheless, when residual lesions were biopsied at 15 day intervals virus was recovered for as long as 120 days, notwithstanding the fact that excessive serous exudation into them occurred regularly and neutralizing antibodies were simultaneously demonstrated in the blood serum.

The present findings confirm our earlier evidence that the curative effect of x-rays on rabbit papillomas (Shope) is the result of direct action on the host's cells rather than on the virus. Indeed, the virus appears to be unaffected by any doses that the host's tissues can endure.

Section B

THE PRODUCTION OF HEPATIC AND GASTRIC LESIONS IN RATS FED A HIGH LEVEL OF HEATED LARD. STUART W. LIPPINCOTT, HAROLD P. MORRIS, and C. DONALD LARSEN. (National Cancer Institute, Bethesda, Md.)

The pathologic changes in the liver and stomach of rats fed a diet containing 35 per cent lard heated to 350° C. for 30 minutes were investigated, because of Roffo's report that adenocarcinoma of the stomach as well as malignant

hepatic tumors developed in rats fed a diet of 50 per cent fat heated to 350° C. for one-half hour. Under our experimental conditions rats began to develop cirrhosis of the liver, after 12 to 18 months, which microscopically appeared as a degenerative and productive hepatitis. Chronic gastric ulcers were found far less frequently. Neither of these two lesions became neoplastic during this time. In a similar period cirrhosis was not observed in Roffo's animals but hepatic and gastric neoplasms were already beginning to appear.

INCREASE IN THE NUMBER AND THE RATE OF GROWTH OF SPONTANEOUS MAMMARY CARCINOMAS CAUSED BY RIBOFLAVIN IN C3H MICE. HAROLD P. MORRIS, STUART W. LIPPINCOTT, and WILLIAM VAN B. ROBERTSON. (National Cancer Institute, Bethesda, Md.)

A synthetic diet deficient only in riboflavin was inadequate to support growth of young mice or maintain the body weight of adult mice. Such a diet, fed to mice with spontaneous tumors, greatly retarded the growth of spontaneous mammary carcinoma. After retardation, the rate of growth could be decidedly stimulated by the addition of riboflavin. A group of mice fed the riboflavin-deficient diet and averaging initially 1.3 breast tumors had an average of 1.5 tumors after 4 weeks, whereas another group on the same diet plus riboflavin increased in 4 weeks from the initial average of 1.2 tumors per mouse to 2.7 tumors. The riboflavin content of the tumor, liver, and muscle of animals with and without riboflavin in the diet will be reported.

INDUCTION OF HEPATOMAS IN MICE BY FEEDING CARBON TETRACHLORIDE. JESSE E. EDWARDS. (National Cancer Institute, Bethesda, Md.)

Published in *J. Nat. Cancer Inst.*, **2**:197-199. 1941; abstract *Cancer Research*, **2**:150. 1942.

THE MITOCHONDRIA AND GOLGI APPARATUS IN THE CELLS OF INDUCED AND SPONTANEOUS HEPATIC TUMORS OF THE MOUSE. ALBERT J. DALTON. (National Cancer Institute, Bethesda, Md.)

Published in *J. Nat. Cancer Inst.*, **2**:565-575. 1942; to be abstracted in *Cancer Research*.

THE USE OF DIETS VARYING IN CALORIES AS A MEANS OF STUDYING THE STAGES OF CARCINOGENESIS. A. TANNENBAUM. (Department of Cancer Research, Michael Reese Hospital, Chicago, Ill.)

It has been shown by the author that caloric restriction inhibits the formation of various types of tumors of the mouse. This phenomenon may be of value in the study of the mechanism of carcinogenesis.

In the experiments here reported tumors were induced by application of benzpyrene solution to the skin, and painting was stopped before tumors appeared in any of the mice. Thus the course of the investigation may be arbitrarily divided into the period of application, and the period in which tumors appeared. The four groups of mice utilized differed only as to their diets, of which two were employed; an ad libitum and a calorie-restricted diet. The first group was fed ad libitum during the period of application of the carcinogen and ad libitum following this period; the second, ad libitum-restricted; the third, restricted-ad libitum; and the fourth, restricted-restricted. A comparison of the number of tumors in the four groups throws light on the mechanism of a calorie-restricted diet in inhibiting neoplasia and, more broadly, on the view that carcinogenesis is polyphasic in nature.

SOME OBSERVATIONS ON THE MECHANISM OF SPECIES ADAPTATION TO CARCINOGENS. R. R. SPENCER and M. B. MELROY. (National Cancer Institute, Bethesda, Md.)

Three widely separated species have been exposed *continuously* to carcinogenic and other agents over a considerable period of time and throughout many cycles of cell division.

By means of lantern slides data are presented to show that:—

1. A given environment, harmless to the individual, may be fatal to the species provided that exposure is *continuous*.

2. On the other hand, when the exposure is *rhythmic* a species can successfully adjust to the environment that is fatal when the exposure is continuous.

The possible broad biological significance of a *rhythmic* rather than a *continuous* exposure of a species to harsh environments is discussed.

EFFECT OF DIETARY CYSTINE ON THE PRODUCTION OF HEPATIC TUMORS BY THE ORAL ADMINISTRATION OF *p*-DIMETHYLAMINOAZOBENZENE. JULIUS WHITE and JESSE E. EDWARDS. (National Cancer Institute, Bethesda, Md.)

Published in *J. Nat. Cancer Inst.*, **2**:535-538. 1942; to be abstracted in *Cancer Research*.

THE EFFECT OF A DIET RELATIVELY LOW IN CYSTINE ON THE PRODUCTION OF SPONTANEOUS BREAST TUMORS IN VIRGIN C3H MICE. JULIUS WHITE and HOWARD B. ANDERVONT. (National Cancer Institute, Bethesda, Md.)

Virgin C3H female mice 4 weeks old were divided into two groups. One was fed a relatively low protein-low cystine diet and the other a relatively low protein-high cystine diet. Litter mate controls were used throughout the experiment. At the end of 10 months all the mice on the high cystine diet had developed spontaneous mammary tumors, whereas none on the low cystine diet had developed them at the end of 13 months.

EFFECT OF CERTAIN DIETARY CONSTITUENTS ON THE INCIDENCE OF LEUKEMIA PRODUCED BY METHYLCHOLANTHRENE IN DILUTE BROWN MICE. JULIUS WHITE and G. BURROUGHS MIDER. (National Cancer Institute, Bethesda, Md.)

It has been previously demonstrated that dilute brown

mice ingesting a diet relatively low in protein but high in cystine will develop leukemia to the extent of 95 per cent when painted with an ether solution of methylcholanthrene. Litter mate controls under similar conditions, but on a low cystine diet, will develop leukemia to the extent of 10 per cent. In this report a study was made to determine the effect of added protein (but low in cystine) to the low cystine diet. The data show that gelatin, as a source of protein low in cystine, failed to increase notably the incidence of leukemia. Furthermore, mice placed on diets adequate in cystine but low and high, respectively, in lysine, all developed leukemia to the same extent as did animals on the high cystine diet reported earlier. These results indicate that the production of leukemia in dilute brown mice painted with methylcholanthrene is dependent on the available sulfur-containing amino acids in the diet, which act in a manner independent of the function of these amino acids to promote growth.

PROTECTION AGAINST THE CARCINOGENIC ACTION OF *p*-DIMETHYLAMINOAZOBENZENE BY LIVER AND YEAST FRACTIONS. K. SUGIURA, C. J. KENSLER (by invitation), and C. P. RHOADS. (Memorial Hospital, New York, N. Y.)

The addition of 10 per cent liver and 15 per cent yeast to a brown rice-carrot diet containing 0.06 per cent *p*-dimethylaminoazobenzene has previously been found to afford complete protection for more than 250 days against the formation of hepatic tumors in the rat. The material in this report covers an attempt to fractionate and concentrate the protective factor or factors in these two materials. The liver fractions were obtained from Dr. G. H. A. Clowes, of Eli Lilly and Co., and several of the yeast fractions from Dr. C. N. Frey, of the Fleischmann Laboratories.

It has been found that a 70 per cent alcohol extract of liver when fed at a 2 equivalent level (1 eq.= 10 per cent liver) afforded definite protection when added as a single supplement to the diet of brown rice-carrot-*p*-dimethylaminoazobenzene. At a 0.4 eq. level not much protection was conferred. An alcohol (70 to 84 per cent) soluble fraction at a 3 eq. level did not protect noticeably, though another alcohol (84 to 93 per cent) soluble fraction at a 3 eq. level afforded considerable protection. A purified liver extract (anti-pernicious anemia fraction) gave distinct protection at a 2 eq. level and very little at a 0.5 per cent eq. level.

An alcohol extract (1.5 eq.) of yeast (1 eq.= 15 per cent yeast), when added to an 18 per cent casein-brown rice-carrot-*p*-dimethylaminoazobenzene diet, exerted considerable, an aqueous extract (1.5 eq.) some protection. An ether extract (1.5 eq.) of the residue from the aqueous extractions, and the final aqueous ether-extracted residue (1.5 eq.) failed to afford significant protection.

As has been previously reported, the addition of riboflavin and casein (18 per cent) to the brown rice-carrot-*p*-dimethylaminoazobenzene diet offered significant protection at 150 days. The substitution of 400γ of riboflavin in the form of a crude concentrate (solvamine) in place of the pure riboflavin failed to protect. It was found also that the addition of a small amount (0.3 eq.) of the 84 to 93 per cent alcohol-soluble liver fraction to a highly protective diet (riboflavin, casein, cystine, choline, brown rice, carrot, *p*-dimethylaminoazobenzene) broke down this protection. As has been recently reported by du Vigneaud, Spangler, Burk, and our group, crystalline biotin also has been found to break down this protection. Analysis of these two fractions for biotin by Dr. Burk indicated that the amount in these two daily supplements was less than 0.05γ.

BIOTIN-AVIDIN BALANCE IN *p*-DIMETHYLAMINOAZOBENZENE TUMOR FORMATION. DEAN BURK, JULIET M. SPANGLER (by invitation), VINCENT DU VIGNEAUD (by invitation), CHARLES KENSLER (by invitation), K. SUGIURA, and C. P. RHOADS. (National Cancer Institute, Bethesda, Md., Cornell University Medical College, and Memorial Hospital, New York, N. Y.)

Biotin (vitamin H, or anti-egg white injury factor) has been shown to exert a pronounced procarcinogenic effect when fed with *p*-dimethylaminoazobenzene to rats maintained on a diet that was otherwise highly protective against tumor formation by virtue of its casein, riboflavin, cystine, choline, and other possible supplements, with or without rice. The hepatic tumor incidence at 150 to 210 days was increased from zero to over 50 per cent upon addition of either pure crystalline biotin or nearly pure biotin ester at levels of 0.3 to 2 γ per rat per day (du Vigneaud, Spangler, Burk, Kensler, Sugiura, and Rhoads, *Science,* **95**:174. 1942).

A study has been made of the hepatoma incidence, tissue biotin content, and comparative histopathology of two groups of rats fed excess biotin on the one hand and excess avidin on the other. For the latter group the diet employed, which was highly protective in the absence of added egg white, contained powdered egg albumen (10 per cent), vitamin-free casein (15 per cent), C.P. sucrose (60 per cent), crisco (10 per cent), O.M. salt mixture (4 per cent), cystine (1 per cent), choline (250 mgm. per 100 cc.), inositol (100 mgm. per 100 cc.), riboflavin (2 mgm. per 100 cc.), additional known vitamins (thiamin, nicotinic acid, pyridoxin, pantothenic acid, A, D, E, K), and *p*-dimethylaminoazobenzene (0.1 per cent). The biotin was negligible except for the rather considerable amount in the egg white, which provided approximately 0.8 γ (bound) biotin and 2 units (excess) avidin per rat per day. On this excess avidin diet moderate biotin deficiency symptoms (vitamin H syndrome) were produced. An excess biotin diet was achieved by further addition of 2 γ crystalline biotin, more than sufficient to overcome the (visible) deficiency syndrome.

The hepatoma incidence in the avidin-excess group with the biotin deficiency syndrome was zero at 180 days. In the biotin excess group, which was free of the deficiency symptoms, the carcinoma incidence was 60 per cent, with large tumor nodules of at least 10 to 20 gm. in each affected liver. The biotin content of the tumors formed (0.2 to 0.8 γ per gm. dry weight) was several-fold lower than that of normal livers from which they were derived, in harmony with many hundreds of analyses performed on *p*-dimethylaminoazobenzene tumors in other experimental

series of the past two years, and with results recently reported by West and Woglom. The biotin content of the protected, avidin-excess group averaged one-third (0.5 to 1 γ per gm.) that of the unaffected nontumor lobes in the biotin excess group, which was essentially normal (1.5 to 3.5 γ per gm.). Protection was thus demonstrably correlated with considerably decreased liver biotin, while tumor formation was associated with a normal content; once a tumor formed, however, it was characterized by a rather low biotin content compared to either type of liver, although not exceptionally low compared with many other types of tissue. The full significance of this interesting relationship remains for future determination. In any event, the egg white (avidin) in the diet definitely lowered the liver biotin content. The riboflavin content of the livers in both the biotin excess and avidin excess groups was essentially the same, and almost normal, namely about 100 γ per gm.

The skin (ventral surfaces) of rats in the avidin excess group, with the vitamin H deficiency symptoms, averaged one-third to one-half (0.02 to 0.05 γ per gm.) that of the biotin-excess, normal appearing, tumor-bearing group (0.07 to 0.08 γ per gm.). Unlike the liver and skin, however, where either tumors or deficiency symptoms respectively were involved, the average biotin content of the spleen (0.2 to 1 γ per gm.), kidney (1.5 to 3 γ per gm.), feces (4 to 8 γ per gm.), urine (0.2 to 0.4 γ per cc.), and blood (0.015 to 0.035 γ per cc.) was practically the same for both groups, although differences would presumably have existed had a greater concentration of egg white in the diet been employed and more than a moderate syndrome obtained.

In both groups the biotin excretion in the feces alone was considerably greater than the dietary intake, due to synthesis in the intestine, followed by complete or nearly complete fixation by the dietary avidin, and then excretion. The feces of rats not fed avidin is ordinarily several-fold lower in biotin than in those receiving this fixative. Net synthesis of biotin occurred mainly in the large intestine or cecum, as indicated by an abrupt rise in biotin content (4 to 8 γ per gm.) as compared with any cephalad sections of the intestinal tract, where the biotin content of the materials therein was less than 1 γ per gm. dry weight.

The biotin excess group was characterized toward the end of the experiment by a heavy coating over the whole body and hair of a very sticky, reddish-brown material, from which a porphyrin has been extracted and tentatively identified by Dr. K. Dobriner as almost certainly protoporphyrin. For all the highly protected animals in the avidin-excess group, Dr. J. E. Edwards reported an almost total absence of the yellowish, iron-free pigment (de-ironized hemosiderin?) ordinarily found in the cortical cells of the kidneys of all *p*-dimethylaminoazobenzene-treated animals not protected against tumor formation, including all those of the biotin excess group whether they had yet developed tumors or not.

In another series of animals kept on avidin excess and biotin excess diets simultaneously with the series described above, but also given 0.05 per cent *p*-aminobenzoic acid, Dr. Edwards reported also dilatation of the lumen in the cortical tubules of the kidney, which did not occur in the absence of the *p*-aminobenzoic acid. None of the animals in the biotin excess group developed tumors in the presence of the *p*-aminobenzoic acid, suggesting an anticarcinogenic action by this substance under the circumstances concerned; further work will be required to establish this with certainty.

THE INFLUENCE OF CALORIC RESTRICTION ON OVARIAN FUNCTION IN RELATION TO MOUSE MAMMARY CANCER INCIDENCE. ZELDA B. BALL (by invitation), ROBERT A. HUSEBY (by invitation), and MAURICE B. VISSCHER (by invitation). (Department of Physiology and the Sivertsen Foundation for Cancer Research, University of Minnesota, Minneapolis, Minn.)

Studies previously reported from this laboratory have shown that simple caloric restriction, without deficiency of protein or other known dietary essentials, resulted in reduction of spontaneous mammary cancer incidence in virgin C3H mice from 67 per cent to zero at 18 months of age. In the present study changes in the development of the uterus and mammary glands were found to occur with caloric restriction. The inferences to be drawn from these observations were discussed.

AFTERNOON SESSION—APRIL 1, 1942

SECTION A

THE EXCRETION OF ESTROGENS AND ANDROGENS IN BREAST CARCINOMA UNDER VARIOUS CONDITIONS OF HORMONE ADMINISTRATION. H. C. TAYLOR, JR., and G. H. TWOMBLY. (Memorial Hospital, New York, N. Y.)

Abstract published in *Cancer Research*, **2**:730. 1942. Original article to be published in *Cancer Research*.

STUDIES IN CANCER. VII. ENZYME DEFICIENCY IN HUMAN CANCER. RIGBY C. ROSKELLEY (by invitation), BENJAMIN N. HORWITT (by invitation), and WILLIAM T. SALTER. (Yale University School of Medicine, New Haven, Conn.)

Through the cooperation of surgeons at the New Haven Hospital it has been possible to collect a series of surviving human tissues and embryos and to transmit them rapidly to the laboratory for study of certain intracellular oxidation-reduction enzyme systems. Collateral histological sections were made of the tissues for comparison of histological and biochemical results. The findings indicate that human tumor cells, like those of many animal cancers, exhibit "enzyme-deficiency" as regards the overall activity of the cytochrome and succinoxidase systems. On the basis of such findings it is possible to distinguish normal from neoplastic tissue, and a biochemical classification of malignant tissues might perhaps be formulated on the basis of enzyme deficiency.

The method distinguishes between homologous tissues, normal and malignant, from the same person; *e.g.*, between normal human kidney parenchyma and hypernephroma or sympathicoblastoma. In some instances the

human tumors were inoculated into the anterior chamber of the eye in animals, in cooperation with Dr. H. S. N. Greene, for determination of tumor autonomy. The data from man harmonize with those for the transitional stages in carcinogenesis due to the Shope rabbit papilloma virus or *p*-dimethylaminoazobenzene.

Because vitamins are the prosthetic groups of enzymes the hypothesis that patients with cancer might show deranged vitamin utilization has been tested. To this end, vitamin "tolerance curves" have been studied in cancer patients and in a variety of control patients. These studies to date show two effects: (1) simple thiamin deficiency is prevalent among cancer patients; (2) even in the absence of frank vitamin deficiency, the thiamin "tolerance curve" is distorted in a high percentage of those with frank malignant disease. The mechanism of this distortion is being investigated.

SOME ANOMALIES IN THE OCCURRENCE OF ALKALINE GLYCEROPHOSPHATASE. HELEN QUINCY WOODARD (by invitation). (Memorial Hospital, New York, N. Y.)

An alkaline glycerophosphatase is produced in bone, where it appears to provide a local excess of phosphoric acid for deposition as calcium phosphate. Early observations indicated that this enzyme was present in osteogenic sarcoma, and that tumors forming large amounts of new bone tended to have a higher phosphatase content than poorly mineralized ones. But some highly malignant osteogenic sarcomas had a high phosphatase content, and nevertheless failed to mineralize. When the growth rate of these tumors was reduced by external roentgen therapy the phosphatase content fell, yet these partially inactivated tumors then became able to lay down new bone. Finally, by the use of tracer doses of radioactive phosphorus, it was shown that in many osteogenic sarcomas an inhibitory factor appeared to be present that prevented the utilization of alkaline glycerophosphatase in the storage of phosphorus.

Study of tissues other than bone suggests that this inhibitory factor may be associated with carbohydrate metabolism. Thus, normal muscle contains no alkaline glycerophosphatase. Small amounts of this enzyme have been found in inflamed muscle, and large amounts in myositis ossificans. In the latter radioactive phosphorus is stored rapidly. In cancers of the liver induced in rats by *p*-dimethylaminoazobenzene carbohydrate metabolism is abnormal, and their alkaline glycerophosphatase activity is ten or more times that of noncancerous liver from the same or other animals. It is possible that in these abnormal tissues an alkaline glycerophosphatase is substituted for a normally occurring dephosphorylating mechanism. It is also possible that in nonossifying osteogenic sarcomas the normal alkaline glycerophosphatase of bone is diverted to some abnormal action. The evidence at present is so incomplete that these suggestions cannot be considered as definite hypotheses, but only as indications for further investigation.

THE URINARY EXCRETION OF ESTROGENS AND 17-KETOSTEROIDS IN PREMENOPAUSAL CARCINOMA OF THE BREAST. IRA T. NATHANSON. (Surgical Service and the Laboratories of the Collis P. Huntington Memorial Hospital of Harvard University, Boston, Mass.)

The etiological role of the estrogens in mammary carcinoma in susceptible strains of mice has been well established. As it seemed desirable, therefore, to study the urinary excretion of the sex hormones in young women with carcinoma of the breast, 145 estrogen and 95 17-ketosteroid determinations were made on a total of 16 patients. The daily excretion of the estrogens and 17-ketosteroids did not vary significantly from normal.

Even though normal total values were found, this is no proof that excretion levels were normal before the development of the carcinoma; or that the individual components of the estrogens and 17-ketosteroids existed in a normal relationship. Therefore these findings do not prove that the sex hormones are not involved in the etiology of the disease, but they do indicate normal total values in spite of the presence of carcinoma during the period of active ovarian function.

STUDIES OF THE EXCRETION OF STEROID IN URINE OF NORMAL PERSONS, CANCER PATIENTS, AND PATIENTS WITH ADRENAL HYPERPLASIA. K. DOBRINER, E. GORDON (by invitation), and C. P. RHOADS. (Memorial Hospital, New York, N. Y.)

Because of the possible interrelation between disturbed sterol metabolism and neoplasia, the excretion of neutral steroids in the urine of 6 normal persons, 8 cancer patients, and 4 patients with adrenal hyperplasia was compared.

The methods for the separation and isolation of the compounds were discussed. A number of new compounds were isolated.

In a quantitative study of the excretion of the alpha-ketosteroids of each urine collection, four groups of compounds were separated and their ratios compared. A constant ratio existed in normal urine whereas in that of patients with cancer and adrenal hyperplasia both quantitative and qualitative differences were found.

Two unsaturated hydrocarbons were demonstrated spectroscopically in the neutral fraction of certain urines.

SKELETAL INVOLVEMENT IN CARCINOMA OF THE CERVIX. BJARNE PEARSON and MANUEL GARCIA. (Departments of Pathology and Bacteriology and Radiology of the School of Medicine of Louisiana State University and Charity Hospital of Louisiana, New Orleans, La.)

Skeletal involvement from carcinoma of the cervix uteri is infrequent. In 74 cases observed at necropsy at Charity Hospital of Louisiana at New Orleans in an eleven year period ending December 31, 1940, 4 with bone involvement were encountered, and in 805 patients treated by the hospital's Radiation Therapy Department during the 3 year period ending December 31, 1941, 3 were observed.

These 7 cases form the basis of this study. In these the following parts of the skeleton were involved: femur, ribs, and vertebrae, 2 instances each; ilium, pubis, and skull, 1 each.

To study the relative distribution of bone lesions all the 98 cases in the available literature were assembled to which our own were added. In these 105, of which 43 came to necropsy, involvement of 150 individual bones was noted. The metastases were thought to be hematogenous in 60 (40 per cent) and by direct extension or lymphogenous in 90 (60 per cent).

In order of frequency the following sites were involved by direct or lymphogenous extension: pelvis 58, vertebrae 32; and by the hematogenous route: lower extremity 37, skull 7, upper extremity 8, ribs 4, sternum and scapula 2 instances each.

One-half of the bone involvement occurred during the first and three-fourths during the second year of the disease.

THE MALIGNANT MELANOMA. E. M. BURKE and A. A. THIBAUDEAU. (State Institute for the Study of Malignant Diseases, Buffalo, N. Y.)

Malignant melanoma is a highly malignant tumor, only 13.9 per cent of our series having survived more than five years. The majority of the growths were pigmented round cell neoplasms. In this series, however, the best results were obtained in nonpigmented round cell tumors. Incomplete removal followed by recurrence usually resulted in rapid metastasis and the death of the patient. Malignant lesions arising from pre-existing growths of long standing were not as fatal as those arising spontaneously a short time only before recognition.

THE RELATION OF RADIOACTIVE PHOSPHORUS TO THE TREATMENT OF MALIGNANT NEOPLASMS. JOHN M. KENNEY. (Memorial Hospital, New York, N. Y.)

Abstract not available.

HUMAN LYMPH NODES OF HODGKIN'S DISEASE GROWN IN TISSUE CULTURE. C. G. GRAND (by invitation) and GLADYS CAMERON (by invitation). Washington Square College, New York University, New York, N. Y.)

The material was obtained by biopsy and tissue cultures were prepared in the usual way. The outgrowth, which was luxuriant, consisted of lymphocytes, granulocytes, fibrocytes, and giant cells. The presence of the last was used as the criterion for identifying the source of the cultures as Hodgkin's disease. In preparations cultured from 10 to 14 days the giant cells enlarged greatly with considerable multiplication of their nuclei.

1. The cultures were stained for virus inclusion bodies, by the technics found to stain virus bodies in tissue cultures inoculated with blood filtrates of measles, scarlet fever, and bovine mastitis, but no indication of inclusions was found.

2. Bacteriological staining methods, including those specific for tubercle bacilli, failed to demonstrate any microorganism.

3. Normal lymph nodes were grown also in media containing serum from patients with Hodgkin's disease, but the results were negative.

These investigations offer no evidence to support the virus or bacterial origin of Hodgkin's disease.

Section B

CARCINOGENESIS AND THE INHIBITION OF SUCCINIC DEHYDROGENASE BY *p*-DIMETHYLAMINOAZOBENZENE DERIVATIVES. V. R. POTTER and K. P. DuBOIS (by invitation). (McArdle Memorial Laboratory, University of Wisconsin, Madison, Wis.)

Compounds which have been shown to arise *in vivo* following the ingestion of *p*-dimethylaminoazobenzene and related compounds are powerful inhibitors of succinic dehydrogenase. The compounds studied include *p*-phenylenediamine, *p*-dimethylaminoaniline, and various methyl derivatives of these two compounds. In addition, quinone, naphthoquinone, and 2-methyl naphthoquinone (artificial vitamin K) are toxic. The results thus far lend support to the work of Kensler who has correlated the carcinogenicity of the parent azo dyes with the toxicity of the diamines to certain yeast enzymes and with the stability of the free radicals which result from the half oxidation of the diamines.

Succinic dehydrogenase is believed to contain a sulfhydryl group and the results with the listed inhibitors as well as with other unrelated inhibitors such as copper and selenite and with various dyes suggest that the enzyme in its active form may be a free sulfhydryl radical En-S which can be reduced to En-SH by succinate, and oxidized back to the free radical by an intramolecular electron shift which reduces a hypothetical prosthetic group. Inhibition of succinic dehydrogenase by *p*-phenylenediamine, for example, could be explained by assuming a combination between the diamine-free radical and the enzyme radical.

The inhibition of succinic dehydrogenase by the *p*-dimethylaminoazobenzene derivatives may be regarded as a step in carcinogenesis or it may simply be a type reaction for a number of other sulfhydryl enzymes which may be involved in the carcinogenic process. The former possibility may be correlated with the reported deficiency of succinic dehydrogenase in tumor tissue.

THE RIBOFLAVIN CONTENT OF TUMOR TISSUE. WILLIAM VAN B. ROBERTSON (by invitation) and HERBERT KAHLER (by invitation). (National Cancer Institute, Bethesda, Md.)

Published in *J. Nat. Cancer Inst.*, **2**:595-600. 1942; to be abstracted in *Cancer Research*.

CHEMICAL AND IMMUNOLOGICAL PROPERTIES OF CONJUGATES PREPARED FROM PROTEINS AND CARCINOGENIC HYDROCARBONS. H. J. CREECH, R. N. JONES, and F. S. CHEEVER (by invitation). (University of Maryland, College Park, Md., and Harvard University, Cambridge, Mass.)

Conjugates have been prepared by the interaction of several proteins with the isocyanates of various polycyclic aromatic hydrocarbons in an aqueous dioxane medium. The degree of conjugation has been determined by spectrophotometric methods and studies have been made of the effect of the composition of the reaction mixture on the extent of the conjugation.

Serum albumins have been found to be the most satis-

factory proteins from the standpoint of the successful introduction of a large number of hydrocarbon prosthetic groups. Other proteins, such as egg albumin and the serum globulins, are so readily denatured that only a small number of hydrocarbon groups may be introduced into the intact protein molecule.

The serum albumin conjugates containing as many as 30 hydrocarbon groups per molecule are soluble in distilled water to an extent of more than 5 per cent, forming optically clear solutions. These conjugates are precipitated from solution at lower concentrations of ammonium sulfate than the unmodified serum albumins and an almost quantitative separation of the substances can be effected by fractional precipitation.

Unless the number of introduced hydrocarbon groups exceeds one-fourth of the theoretical, no serological prosthetic group specificity is evident. This preliminary observation has limited the main immunological investigations to the conjugates prepared from serum albumins. The horse serum albumin conjugates have been found to be the best antigens for the purposes of immunization and bovine serum albumin conjugates are the best test antigens. Although the method of conjugation has not destroyed the "albumin" specificity (all antisera produced by the injection of horse serum albumin conjugates react serologically with test solutions of unmodified horse serum albumin), there is a strong serological reaction directed towards the hydrocarbon prosthetic group as demonstrated, for example, by the serological reaction between the antiserum from 1,2-benzanthryl-10-carbamido horse serum albumin and the test antigen 1,2-benzanthryl-carbamido bovine serum albumin. The titer at which this reaction occurs is influenced considerably by the amount of combined hydrocarbon in the particular conjugate under investigation; the higher the content, the stronger is the reaction. The prosthetic group activity is not directed exclusively towards the homologous hydrocarbon because a certain degree of overlapping has been noted between 1,2-benzanthracene and 3,4-benzpyrene.

The intense fluorescence in ultraviolet light exhibited by the conjugates has promoted investigations utilizing this "tracer" characteristic both in immunological and biochemical studies.

THE SPECTROGRAPHIC ANALYSIS OF CARCINOGENIC HYDROCARBONS AND CONJUGATED PROTEINS. R. NORMAN JONES and HUGH J. CREECH. (Chemical Laboratory, Harvard University, Cambridge, Mass., and Department of Chemistry, University of Maryland, College Park, Md.)

The spectrographic methods used for the control and analysis of the hydrocarbon-carbamido proteins discussed in the previous paper are described.

It is known that polynuclear aromatic hydrocarbons of the type used in this work possess characteristic absorption spectra and evidence is presented in support of the statement that the introduction of the carbamido linkage does not produce any pronounced change in the absorption spectrum of the hydrocarbon. This is true whether the carbamido linkage is associated with a protein or with an acetic acid molecule as in 1,2-benzanthryl-10-carbamidoacetic acid.

Observations of the spectra of proteins of the type used in this work show that they absorb light to any significant extent only if the wave length is less than 3,200 Å, so that, in the spectra of the conjugated proteins, all light absorption at wave lengths greater than 3,200 Å can be attributed to the effect of the attached hydrocarbon group.

If the assumption is made that the hydrocarbon-carbamido group has the same intensity of absorption when attached to acetic acid as it does when attached to a protein molecule, the extent of conjugation can be calculated from the absorption data and is usually expressed as milligrams per gram of protein.

The use of this technic for the determination of the purity of the protein conjugates and freedom from adsorbed impurities is discussed; certain assumptions involved in the quantitative treatment are mentioned; and some evidence based on analyses of known mixtures of 1,2-benzanthryl-10-carbamidoacetic acid and horse serum albumin is presented in support of the accuracy of the method.

THE INHIBITION OF LIVER OXIDATIVES BY SPLIT PRODUCTS OF *p*-DIMETHYLAMINOAZOBENZENE. C. J. KENSLER and C. P. RHOADS. (Memorial Hospital, New York, N. Y.)

The two azo carcinogens whose metabolism by the rat has been studied have been found to be split *in vivo* at the azo linkage. One metabolite of *p*-dimethylaminoazobenzene, *p*-phenylenediamine, and a probable intermediary metabolite, N,N-dimethyl-*p*-phenylenediamine, have been found to inhibit fermentation in a washed yeast system in which the limiting factor is diphosphopyridine nucleotide. This inhibition can be prevented either by keeping the two aromatic diamines in the reduced state or by adding a large amount of the diphosphopyridine nucleotide. These two compounds have also been found to inhibit a yeast carboxylase preparation. This inhibition also can be prevented by keeping the aromatic diamine reduced and by adding an excess of the limiting coenzyme, in this case cocarboxylase. The addition of large amounts of the nonfunctioning or nonlimiting coenzyme did not protect either system. From these studies it was concluded that the inhibitions were caused by an oxidative inactivation of protein catalysts.

As *p*-phenylenediamine and N,N-dimethyl-*p*-phenylenediamine are probably formed from the *p*-dimethylaminoazobenzene molecule in the liver their effect on liver slice and brei respiration was studied. It was found that the oxygen consumption of both liver slice and brei (200 mgm.) was inhibited. The N,N-dimethyl-*p*-phenylenediamine was more toxic than *p*-phenylenediamine. When larger amounts of the brei were used it was found that the percentage inhibition caused by N,N-dimethyl-*p*-phenylenediamine was decreased. This compound was toxic not only to liver but also to other tissues tested, namely: spleen, kidney, brain, and testis. Respiration of slices of *p*-dimethylaminoazobenzene liver cancer was also found to be inhibited, although slightly less than normal liver.

Oxygen consumption of *p*-dimethylaminoazobenzene tumor brei was not inhibited.

The cytochrome oxidase system (liver brei and heart muscle preparation) was not inhibited by equivalent concentrations (1×10^{-4} to 1×10^{-3} *M*). However, this system was inactivated by higher concentrations (2×10^{-2} *M*). The addition of cytochrome *c* failed to protect or restore activity. The succinoxidase system (liver brei and heart muscle preparation) was found to be sensitive to inactivation by low concentrations of N,N-dimethyl-*p*-phenylenediamine and by *p*-phenylenediamine (1×10^{-4} to 1×10^{-3} *M*). This system was also inhibited by these compounds (1×10^{-3} *M*) when liver slices were incubated for 15 minutes before sodium succinate was tipped into the medium.

The succinoxidase system has been found by Salter and his coworkers to decrease rapidly in the liver of rats fed *p*-dimethylaminoazobenzene. These investigators have also found that the hepatic tumors produced have low succinoxidase activity. Potter and his coworkers have found that the succinoxidase falls in the livers of rats fed a riboflavin deficient diet. Work in our laboratories has shown that the addition of riboflavin and casein to a brown rice-carrot-*p*-dimethylaminoazobenzene diet affords considerable protection against liver damage and cancer formation in rats. These experiments offer a possible explanation of the riboflavin effect. Bernheim and his coworkers have previously reported that aminophenol, another metabolite of *p*-dimethylaminoazobenzene, inhibits xanthine oxidase, which is believed to be a flavoprotein.

Xanthine oxidase (liver brei) has been found to be slightly inhibited by N,N-dimethyl-*p*-phenylenediamine. Uricase (liver brei preparation) and *d*-amino acid oxidase (kidney preparation) were not inhibited by these compounds. Alkaline and acid phosphatase has been found by Dr. Woodard not to be inactivated by N,N-dimethyl-*p*-phenylenediamine.

PROTEOSE CHANGES IN RAT BLOOD INDUCED BY TRANSPLANTABLE AND PRIMARY TUMORS. RICHARD J. WINZLER (by invitation) and DEAN BURK. (National Cancer Institute, Bethesda, Md.)

Polarographic studies with the blood of tumor-bearing rats have demonstrated the presence of a nondialyzable, heat-stable, cystine-containing material that may be precipitated by saturated ammonium sulfate, alcohol, or tungstic, metaphosphoric, or phosphotungstic acid, but not by trichloracetic or sulfosalicylic acid. Normal rat blood contained much less of this material with proteose properties. The blood of tumor-bearing rats contained from 20 to 50 mgm. of proteose-N per 100 cc., whereas normal blood contained 3 to 4 mgm.

During the growth in young rats of transplanted hepatomas from a diameter of about 0.2 to 2 cm. over a period of 3 to 30 days after inoculation, there was a continual increase in the blood proteose concentration up to a level of eightfold to tenfold higher than the normal. Similar increases were obtained with the Jensen rat sarcoma. Primary hepatomas induced by the feeding of *p*-dimethylaminoazobenzene caused threefold to tenfold increases. No definite influence of strain, sex, age, or diet on the filtrate wave of normal or tumor-bearing rats has yet been observed. When transplanted hepatoma 31 was inoculated, particularly into older rats, it frequently grew to a diameter of 1 to 2 cm. and then rapidly regressed. During the growth period there was the usual proteose increase, which was followed by a rapid return to normal as the tumor regressed. Extirpation of well established hepatoma No. 31 tumors was followed by a return of blood proteose to normal or slightly higher than normal values within a few days.

Information as to the cause and source of the increased proteose in the blood of tumor-bearing animals was afforded by other experiments. Neither rapid regenerative growth of liver following partial hepatectomy, nor development of liver cirrhosis led to increased blood proteose. Fetal growth caused a definite increase in the blood proteose of the mother only during the final days before term. The subcutaneous or intraperitoneal injection of large amounts (1 gm.) of homogenized healthy tumor or necrotic tumor tissue had no effect on the blood proteose level. Pyogenic infections in the rat, as in man and dog, and also foreign body reactions, led to increased blood proteose. Large transplanted liver tumors irradiated with x-ray doses of 4,000 r ceased growing, but there was no decrease in blood proteose from the high level occurring before irradiation for at least 12 days.

Whether or not the increase in the proteose content of cancer blood represents split proteins arising from blood protein, tumor protein, or necrotic tumor tissue remains to be definitely determined. The degree of necrosis seems to be unimportant in influencing the magnitude of the blood changes. The quantitative extent of proteose formation often appears to exceed the total amount of tumor protein, but never to exceed the concurrent blood albumin disappearance. These facts, together with the similarity in the proteose changes resulting from cancer and from foreign body reactions, make it seem likely that the proteoses originate primarily from the blood protein, or indirectly from tissues other than the tumor itself.

Parallel studies with normal and pathological blood, and blood from cancer patients, are being carried out.

PERMEABILITY AND METABOLISM OF EMBRYONIC AND MALIGNANT CELLS IN CULTURE. W. E. COHN and A. M. BRUES. (Collis P. Huntington Memorial Hospital of Harvard University, Boston, Mass.)

With a tissue culture method developed by the authors, the uptake of radioactive phosphorus and potassium by embryonic and malignant tissues *in vitro* has been studied without disturbance of the environmental conditions conducive to life and growth of the cells. In the case of phosphorus (supplied as inorganic phosphate in the medium) chemical analysis of tissue and mathematical analysis of uptake curves enable us to dissociate permeability and metabolic turnover and express each in terms of mathematical rate constants following the general formula $A=1-E^{Kt}$. Results indicate that the permeability constants of chick embryo muscle, mouse embryo muscle, and mouse carcinoma and sarcoma to phosphate are all of the

same order of magnitude, representing around 30 to 80 per cent exchange across the cell surface per hour. Metabolic turnover constants are much lower, ranging from 0.5 to 1 per cent per hour, or close to the observed growth rates of the tissues under cultivation. Permeability to potassium gives constants slightly higher than those for phosphate permeability, in both normal and malignant tissues.

RELATION OF NUCLEIC ACID METABOLISM TO GROWTH. A. M. BRUES, M. M. TRACY, and W. E. COHN. (Collis P. Huntington Memorial Hospital of Harvard University, Boston, Mass.)

The uptake of radioactive phosphorus by various chemical fractions of resting and regenerating rat liver, including isolated nucleic acids, has been studied in order to find whether the recognized biological stability of chromosomes is associated with chemical stability of chromosomal constituents. The results show that the nucleic acid isolated has a much slower uptake of phosphorus than any other phosphorus fraction obtained from the resting organ, while in the regenerating organ the uptake corresponds in amount to the amount of new nucleic acid formed in growth. Other fractions of phosphorus show a rapid uptake (due to rapid simultaneous synthesis and degradation of the molecules) that is not altered with growth. We have shown that the relatively rapid turnover in the "nucleoprotein" fraction reported by other workers is due to turnover of phosphorus which is in other forms than the nucleic acids isolated from tissue. This apparent irreversible nature of nucleic acid synthesis, in contrast to the reversibility of many intracellular reactions, suggests the possibility that nucleic acids may act as pacemakers in the growth process, an hypothesis which would account for the relatively high nuclear size and nucleic acid content of tumors.

TRANSAMINATION IN LIVER FROM RATS FED *p*-DIMETHYLAMINOAZOBENZENE. PHILIP P. COHEN (by invitation), G. LEVERNE HEKHUIS (by invitation), and EVA K. SOBER (by invitation). (Laboratory of Physiological Chemistry, Yale University, New Haven, Conn., and the Department of Physiological Chemistry, University of Wisconsin, Madison, Wis.)

Published in *Cancer Research,* **2**:405-410. 1942; abstract p. 522.

METABOLIC STUDIES IN LYMPHOID LEUKEMIA. V. E. HALL (by invitation). (Department of Pathology, Cornell University Medical College, New York, N. Y.)

Published in *Cancer Research,* **2**:411-421. 1942; abstract p. 522.

American Association for Cancer Research, Inc.

35th Annual Meeting

Parker House, Boston, Massachusetts
March 30-April 1, 1942

Proceedings of Business Sessions

Minutes of the Meeting of the Board of Directors Held March 30, 1942

The annual meeting of the Board of Directors was held at the Parker House in the City of Boston on the evening of March 30, 1942.

The meeting convened at 9:00 p. m. with the President, Dr. Carl Voegtlin, in the chair. There were present:—

Directors, H. B. Andervont, H. J. Bagg, C. C. Little, James B. Murphy, B. T. Simpson, George M. Smith, A. A. Thibaudeau, Carl Voegtlin, Shields Warren, and William H. Woglom,

forming a quorum.

The president reported that Dr. Murphy had resigned as Chairman of the Journal Committee and that he had been replaced by Dr. C. C. Little.

The minutes of previous meetings (1941) were approved as read.

The Vice-President had no report to make.

The Secretary-Treasurer presented the list of 62 applicants for membership. A list of nine members deceased during the past year was read. Eleven resignations were tendered, four from members who are in the armed forces of the United States of America.

The Treasurer's report was presented and approved.

The Treasurer requested instructions relative to the bill of the Lord Baltimore Press for publishing records of the 1941 meeting in *Cancer Research*. This he was directed to pay.

The result of the ballot for Directors showed out of 220 returns the following number of votes for the various nominees:—

James Ewing	134
Carl Voegtlin	125
Ludvig Hektoen	117
George M. Smith	115
James B. Murphy	112
C. P. Rhoads	110
W. U. Gardner	87
Balduin Lucké	69
M. J. Shear	2

One vote each was cast for:—

James P. Simonds
Stafford L. Warren
H. B. Andervont
John J. Bittner
Samuel Haythorn
John Lockwood
Wm. C. MacCarty, Sr.
H. P. Rusch
Peyton Rous
John J. Morton
George T. Pack
Hayes E. Martin
Cushman D. Haagensen

As Dr. Ewing declined to accept the nomination, the Board of Directors decided to recommend the election of the four nominees receiving the next largest number of votes, to wit: Carl Voegtlin, Ludvig Hektoen, George M. Smith, and James B. Murphy.

The activities of the Program Committee were reported by Dr. C. C. Little, *Chairman*.

For the Journal Committee, reports were made by Dr. C. C. Little and Dr. James B. Murphy. The Secretary was directed to write Dr. S. Bayne-Jones and Dr. William H. Woglom commending them for their work in furthering the interest of the journal, *Cancer Research*. It was suggested that it would be desirable for the Board of Directors to have a report on the financial state of the journal.

The report of the Membership Committee was made by Dr. William H. Woglom, *Chairman*. He stated that the qualifications for all candidates for membership had been reviewed by members of the Committee, which recommended the election of 61 applicants. The board accepted the recommendation of the Committee and elected the following as members of the Association:—

Algire, Glenn H., B.S., M.D., Bethesda, Md.
Angrist, Alfred, B.S., Jamaica, N. Y.
Barnes, W. A., A.B., M.D., New York, N. Y.
Bartfeld, Harry, A.B., M.D., New York, N. Y.
Bennett, Mary A., Ph.D., Philadelphia, Pa.
Brown, Robert L., A.B., M.D., Rochester, N. Y.
Burack, Ethel, B.A., Ph.D., Albany, N. Y.
Cline, Joseph K., M.A., Ph.D., Galveston, Texas.
Cohen, Philip P., Ph.D., M.D., Madison, Wis.
Cole, R. K., B.S., Ph.D., Ithaca, N. Y.
Coombe, Reginald, A.B., Greenwich, Conn.
Cox, Alvin J., Jr., A.B., M.D., San Francisco, Calif.
Crossland, Ruth S., A.B., M.S., Ph.D., Rochester, N. Y.
Dalton, Albert J., B.S., M.A., Ph.D., Rockville, Md.
DuBilier, Benjamin, M.D., C.M., Columbia, Mo.
du Vigneaud, Vincent, Ph.D., New York, N. Y.
Eggers, Harold E., M.A., M.D., Omaha, Neb.
Engel, Lewis L., B.S., Ph.D., Rochester, Minn.
Failla, Gioacchino, E.E., M.A., D.Sc., New York, N. Y.
Fleming, R. M., M.D., Miami, Fla.
Frazell, Edgar L., M.D., New York, N. Y.
Freeman, Smith, Ph.D., M.D., Chicago, Ill.
Goldfeder, Anna, D.Sc., M.A.C., New York, N. Y.
Guzman, Gonzalez, M.D., Mexico, D. F.
Hodes, Philip J., B.S., M.D., Sc.D., Philadelphia, Pa.
Hooker, Charles W., A.B., A.M., Ph.D., New Haven, Conn.
Kaliss, N., A.M., Ph.D., New York, N. Y.
Kensler, C. J., A.B., M.A., New York, N. Y.
Kline, Bernard E., B.S., M.D., Madison, Wis.
Kretchmer, H. L., M.D., Chicago, Ill.

Lund, Charles C., M.D., Boston, Mass.
Meader, Ralph G., Ph.D., New Haven, Conn.
Medinger, Fred C., A.B., M.D., New York, N. Y.
Melroy, Malcolm B., B.S., M.D., Washington, D. C.
Murray, Margaret R., A.B., M.D., Ph.D., New York, N. Y.
Nelson, Warren O., A.B., Sc.M., Ph.D., Detroit, Mich.
Nettleship, Anderson, B.S., M.D., Bethesda, Md.
Newell, Robert R., M.D., San Francisco, Calif.
Olcott, Charles T., A.B., M.D., New York, N. Y.
Pfeiffer, Carroll A., A.B., M.S., Ph.D., New Haven, Conn.
Popper, Hans, M.D., M.S., Chicago, Ill.
Potter, Van Rensselaer, M.D., Ph.D., Madison, Wis.
Riegel, Byron, A.B., M.A., Ph.D., Evanston, Ill.
Rosahn, Paul D., A.B., M.D., New Britain, Conn.
Rosenthal, Otto, M.D., Philadelphia, Pa.
Saphir, Otto, M.D., Chicago, Ill.
Saxton, John A., A.B., M.D., New York, N. Y.
Selye, Hans, M.D., Ph.D., F.R.S., Montreal, Quebec.
Shack, Joseph, A.B., Ph.D., Bethesda, Md.
Stone, Robert S., B.A., M.A., M.B., M.D., San Francisco, Calif.
Stowell, Robert E., A.B., M.D., St. Louis, Mo.
Suntzeff, V., M.D., St. Louis, Mo.
Sutton, Richard L., Jr., A.M., M.D., Kansas City, Mo.
Traut, H. F., B.S., M.D., New York, N. Y.
Truog, Clarence P., B.S., M.B., M.D., Ph.D., Miami, Fla.
White, Edward P., M.D., Ph.D., Jamaica, Mass.
Williams, W. Lane, B.S., M.A., Ph.D., New Haven, Conn.
Winzler, Richard J., B.A., Ph.D., Bethesda, Md.
Wood, David A., A.B., M.D., San Francisco, Calif.
Zahl, Paul A., B.A., M.A., Ph.D., New York, N. Y.
Zeckwer, Isolde T., A.B., M.D., Philadelphia, Pa.

The Secretary reported the resignations of the following members:—

Craig, Francis N., Massachusetts General Hospital, Boston, Mass.
White, Abraham, Yale University, New Haven, Conn.
McFarland, Joseph, Germantown, Pa. Requests emeritus membership.
Lynch, Frances W., St. Paul, Minn.
Scheele, L., National Cancer Institute, Bethesda, Md. War work.
Wartman, W. B., Cleveland, Ohio. In Army.
Lufkin, N. H., Minneapolis, Minn. In Navy.
Jones, Geo. W., Pomona, Calif.
Jones, D. F., New Haven, Conn.
Lawson, John D., Sacramento, Calif.
Callender, Col. Geo. R., Washington, D. C. In Army.

During the past year, reports of the death of the following members have reached the Secretary:—

Mallory, Frank B., Boston, Mass.
Connor, Charles L., San Francisco, Calif.
Sittenfield, Maurice J., New York, N. Y.
Crawford, Baxter L., Wyncote, Pa.
Banting, Sir Frederick, Toronto, Ontario.
Orr, C., Buffalo, N. Y.
Pecher, Charles, Berkeley, Calif.
Kast, Ludwig, New York, N. Y.
Ill, Charles, Newark, N. J.

The Nominating Committee presented the following suggestions for officers of the Association for 1942:—

Shields Warren, *President*
A. A. Thibaudeau, *Vice-President*
W. U. Gardner, *Acting Secretary*

These suggestions were accepted by the Board of Directors. A vote of thanks to the retiring Secretary-Treasurer was moved and approved.

Shields Warren, *Chairman,* reported on the work of the Committee on Local Arrangements for the 1942 meeting, and a vote of thanks was tendered Dr. Warren for his efforts.

It was duly moved and carried that members in military and naval forces have their dues remitted for the period of such service.

After some discussion it was decided on motion duly made and carried that the annual dues be raised from $2.00 to $3.00, of which $1.00 is to be sent to *Cancer Research* as official organ of the Association.

A motion that the Association establish a class of Emeritus Members, exempt from dues, to consist of those who have attained the age of 65 years with 10 or more years of membership, was duly made and carried. This membership is available to those qualified, on application.

Dr. Joseph McFarland of Philadelphia was elected an Emeritus Member of the Association.

Dr. James Ewing of New York was elected an Honorary Member of the Association.

It was duly moved and carried that the last phrase of Article I, Section 2, of the By-Laws be eliminated, this section to read, as amended, "Section 2. Election of Members. The Board of Directors at any time, and from time to time, may elect to membership such persons as they consider eligible."

It was duly moved and carried that stenographic reporting of the annual meeting be dispensed with.

Adjournment was taken at 11:30 p. m.

Carl Voegtlin, *President*
A. A. Thibaudeau, *Secretary-Treasurer*

Minutes of the Meeting of Members Held March 31, 1942

This meeting convened at 9:00 a. m. with Dr. Carl Voegtlin, *President,* in the chair.

The minutes of the 1941 meeting and a resume of the 1942 meeting of the Board of Directors were read by the Secretary. On motion duly made and seconded these minutes were accepted by the meeting.

The election of the officers for 1942 proposed by the Board of Directors was approved by the meeting as was also the increase in the annual dues from $2.00 to $3.00, with $1.00 to go to *Cancer Research* as official organ of the Association.

Elimination of the last phrase in Section 2 of Article I of the By-Laws of the Association was also approved by the members.

The meeting then proceeded to the scientific program.

Carl Voegtlin, *President*
A. A. Thibaudeau, *Secretary-Treasurer*

Minutes of the Meeting of the Board of Directors Held April 1, 1942

The meeting was called to order by President Shields Warren. Directors S. Bayne-Jones, L. Hektoen, G. M. Smith, and B. T. Simpson were absent.

The motion was made, seconded, and carried that W. U. Gardner, *Acting Secretary,* be made *Treasurer* also.

The applications for membership of Drs. W. L. Russell and E. S. Russell, whose nominations were proposed and seconded, were accepted and the applicants made members of the Association.

The proposal was made that papers to be presented at future meetings be introduced by some member, and that it be stated that they are presented under such sponsorship, rather than by invitation, as has been the custom in the past. It was proposed also that the meetings of the Association be extended from two days to three in order to avoid parallel sessions. These questions were referred to the new Program Committee.

It was proposed also that attempts be made to improve the quality of lantern slides, and that to this end specifications for suitable illustrations be given in announcements of meetings.

Report of Committees:—

Journal Committee.—Dr. C. C. Little, *Chairman,* reported that subscriptions to *Cancer Research* to members and non-members of the Association were as follows:—

Number of subscriptions	Subscription rate and status
1941	1941
447	$7.00 non-members
148	$5.00 members
1942	1942
397	$7.00 non-members
146	$5.00 members

Dr. Mildred W. S. Schram's expression of appreciation of the contribution to *Cancer Research* by the Association to the extent of $1.00 per member per year was conveyed. The anticipated decline in the number of papers available was discussed and the opinion prevailed that the journal should be continued with fewer issues per year if necessary. The resignation of Dr. A. V. Winchell, *Business Manager of Cancer Research,* was offered. Dr. Winchell, who is now in the Navy, has served as Business Manager since the journal was founded. His resignation was accepted with regret.

Program Committee.—Dr. C. C. Little, *Chairman,* expressed his appreciation to the members of his Committee, and to Dr. C. P. Rhoads for his special assistance in arranging the evening program.

Membership Committee.—Dr. W. H. Woglom, *Chairman,* had no further report but expressed his appreciation of the assistance of Dr. H. B. Andervont and Dr. S. Bayne-Jones and requested the Secretary to convey his thanks to the latter, who was not present.

New and Unfinished Business

Committees for the year 1942-43 were appointed by President Shields Warren as follows:—

Journal Committee.—C. C. Little, *Chairman;* J. B. Murphy; S. Bayne-Jones.

Program Committee.—C. P. Rhoads, *Chairman;* C. C. Little; W. U. Gardner.

Membership Committee.—W. H. Woglom, *Chairman;* H. B. Andervont; S. Bayne-Jones.

Nominating Committee.—H. B. Andervont, *Chairman;* A. A. Thibaudeau; H. J. Bagg.

The meeting adjourned at 2 p. m.

Shields Warren, *President*
W. U. Gardner, *Acting Secretary-Treasurer*

Abstracts

Reports of Experimental Research

Carcinogenic Compounds

BRIGGS, J. B., and BRIGGS, R. W. [McGill Univ., Montreal, Canada] THE EFFECTS OF A WATER-SOLUBLE CARCINOGEN ON EARLY FROG DEVELOPMENT. Cancer Research, 3:1-12. 1942.

Eggs of the frog, *Rana pipiens,* were reared in solutions of a water-soluble carcinogen in order to determine whether there would be any effect on the rate of development, or on the morphological organization of the embryo. The carcinogen used was Na-1,2,5,6-dibenzanthracene-9,10-endo-α,β-succinate (*trans*). Exposure began 1 hour after fertilization, and the concentrations used were 0.02, 0.213, 2.13, 4.26, and 10.0 mgm. per 100 cc.

The results were: (1) No evidence of stimulative effect in any concentration. (2) Retardation of developmental rate (after beginning of gastrulation) in solutions containing 2.13, 4.26, and 10.0 mgm. per 100 cc., the degree of retardation being proportional to the concentration. (3) Degeneration of embryos in these same three concentrations—degeneration following a definite uniform pattern, and beginning always at the same developmental stage for a given concentration. (4) Differential retardation of development of capacity for muscular movement relative to morphological development. (5) Lack of any effect on the morphological organization of the embryo.

Various control experiments, with a surface tension-reducing agent (aerosol O.T.), Na-succinate, a related noncarcinogen (anthracene-succinate), metabolic poisons, and miscellaneous substances, indicated that the effects noted above were specific for dibenzanthracene-succinate, and suggested further that the carcinogenicity of this chemical might be responsible for the effects. Treatment of eggs with another water-soluble carcinogen, Na-3-methylcholanthrene-6,12b-endo-α,β-succinate, gave confirmatory evidence on this latter point.—Authors' abstract.

COLLINS, V. J., GARDNER, W. U., and STRONG, L. C. [Yale Univ. Sch. of Med., New Haven, Conn.] EXPERIMENTAL GASTRIC TUMORS IN MICE. Cancer Research, 3:29-35. 1943.

Benzpyrene dissolved in sesame oil was administered orally or by intravaginal instillation to 133 mice of 5 inbred strains, namely C3H, NH, C57, A, and CHI, to determine its effect upon the formation of tumors of the gastrointestinal tract. All the resulting gastric tumors arose in the forestomach and were of the squamous cell type. The highest incidence of gastric papillomas and carcinomas occurred in mice of the C3H strain. A high incidence of gastric tumors was observed in mice of the C57 and NH strains and a low incidence of carcinomas in mice of the CHI and A strains. No definite sex difference in the incidence of gastric tumors was noted in the C3H strain. However, the incidence of carcinomas was higher in ovariectomized mice of the C3H and NH strains than in intact mice treated similarly with the carcinogen. Most gastric tumors appeared at the greater curvature of the forestomach, the fundus of which was more frequently involved than the area near the limiting ridge. Tumors occasionally developed on the lesser curvature and on the anterior or posterior wall. Carcinomas and papillomas appeared in 22 and 69 of the treated mice respectively. They were of the squamous cell type showing a mixture of keratinized flat cells, prickle cells, and basal cells. The 22 carcinomas infiltrated the gastric wall, extended to the serosa, and invaded the lymphatics. Papillomas and carcinomas of the skin occurred at the sites contaminated by the benzpyrene in nearly all animals. Whenever a carcinoma of the skin was found the gastric tumor was usually small and benign; on the other hand, mice with carcinoma of the stomach usually had benign skin lesions. A gastric carcinoma from a C57 mouse was successfully transplanted to 3 normal mice of the same strain.—Authors' abstract.

CRAMER, W., and STOWELL, R. E. [Barnard Free Skin and Cancer Hosp. and Washington Univ. Sch. of Med., St. Louis, Mo.] SKIN CARCINOGENESIS BY A SINGLE APPLICATION OF 20-METHYLCHOLANTHRENE. Cancer Research, 3:36-42. 1943.

Cancer can be induced in the skin of mice in response to a single cutaneous application of a potent carcinogen such as 20-methylcholanthrene. In the experiment recorded in this paper 6 of 14 female Swiss mice developed malignant tumors after intervals of 13 to 42 weeks. Five of these malignant neoplasms were squamous carcinomas developing at the site of application. The significance of this phenomenon lies in the fact that methylcholanthrene applied cutaneously disappears from the skin after 1 to 2 weeks. The bearing of these observations on the interpretation of the mode of action of chemical carcinogens is discussed.—Authors' abstract.

LAURIDSEN, J., and EGGERS, H. E. [Univ. of Nebraska Med. Coll., Omaha, Nebr.] CARCINOGENESIS AFTER MULTIPLE IRRITATION. Cancer Research, 3:43-46. 1943.

An attempt was made to determine whether carcinogenesis is facilitated by the additive effect of diverse irritations. Approximately 100 mice were taken from each of two inbred strains, one with a low tumor incidence and the other with a high incidence of spontaneous mammary tumors. Half the animals in each group were subjected to cutaneous applications of 1,2,5,6-dibenzanthracene and to repeated light cauterization, the other half to dibenzan-

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thracene only. Cutaneous and subcutaneous tumors developed more rapidly in the cauterized animals. This effect was shown more decidedly by the animals from the low tumor strain. In the mice from the high tumor strain, the higher incidence of induced tumors among the cauterized mice was largely due to the greater number of sarcomas that developed rather than to induced skin tumors. It is suggested that in these animals the higher incidence of sarcomas may be an expression of a constitutional predisposition that is masked in the skin by some accessory factor.—Authors' abstract.

Hormones

HEIMAN, J. [Coll. of Physicians and Surgeons, Columbia Univ., New York, N. Y.] COMPARATIVE EFFECTS OF ESTROGEN, TESTOSTERONE, AND PROGESTERONE ON BENIGN MAMMARY TUMORS OF THE RAT. Cancer Research, 3:65-69. 1943.

Abstracted in the Report of the Endocrine-Cancer Conference, Cancer Research, **2**:726. 1942.

Genetics

BURDETTE, W. J., and STRONG, L. C. [Yale Univ. Sch. of Med., New Haven, Conn.] THE INHERITANCE OF SUSCEPTIBILITY TO TUMORS INDUCED IN MICE. I. TUMORS INDUCED BY METHYLCHOLANTHRENE IN FIVE INBRED STRAINS OF MICE. Cancer Research, 3:13-20. 1943.

The susceptibility to neoplasms induced with a single subcutaneous injection of methylcholanthrene was determined for 5 inbred strains of mice. Differences in the time of appearance of tumors were found. The predominant type of growth was rhabdomyosarcoma in some strains and spindle cell sarcoma in others. The survival time of mice of the several strains was not always the same and did not necessarily parallel their susceptibility to induced tumors.—Authors' abstract.

STRONG, L. C., COLLINS, V. J., and DURAND, E. A. [Yale Univ. Sch. of Med., New Haven, Conn.] A GENETIC ANALYSIS OF THE INDUCTION OF TUMORS BY METHYLCHOLANTHRENE. IV. THE PROBABLE REMOTE INDUCTION OF VARIOUS TYPES OF GASTRIC LESIONS. Cancer Research, 3:21-28. 1943.

A variety of gastric lesions, including glandular hyperplasia, adenoma, adenocarcinoma or potential adenocarcinoma, squamous cell papilloma, and squamous cell carcinoma have been obtained in mice of the NHO strain after subcutaneous injection, at 60 days of age, of 1 mgm. of methylcholanthrene dissolved in 0.1 cc. of sesame oil.

It appears, although it is not definitely proved, that the presence of the carcinogen may have had some influence on the appearance of these lesions.

The gastric lesions occurred in mice that had also developed adenocarcinoma of the lung or spindle cell sarcoma, or quite independently of any other form of lesion.—Authors' summary.

Biochemistry and Nutrition

CHALKLEY, H. W. [Nat. Cancer Inst., Bethesda, Md.] EFFECT OF CERTAIN SULFUR-CONTAINING COMPOUNDS ON THE INITIATION OF MITOSIS IN AMOEBA PROTEUS. J. Nat. Cancer Inst., 2:425-447. 1942.

A method was devised to permit detection and measurement of rapid changes in the rate of entry into mitosis in *Amoeba proteus*. Sulfur-containing organic compounds were added in high dilution (2×10^{-4} to 5×10^{-3} mols per liter) to cultures of selected amebae, and their effect on the number of cells entering prophase as a function of time was studied. Methionine and cysteic acid gave negative results. Cystine, cysteine, glutathione, cystine disulfoxide, β,β′-dithiodipropionic acid, cystinyldiglycine, and a mixture of glutamic acid and glycine all affected the entrance of the ameba into mitosis.

The effects obtained were: (1) a sudden increase or decrease in rate, and (2) a change from one level of constant acceleration to another. The effect of each compound studied was generally a mixture of these two results. The most effective reagents used were those containing sulfur in labile form as –SH– and –SS–. These reagents in general exerted a stimulative action but cysteine, β,β′-dithiodipropionic acid, and cystinyldiglycine exerted partial or total depressive actions. It is concluded that the presence of labile sulfur alone does not insure a stimulative action. Seventeen figures showing forms of ameba and control and experimental curves are included.—F. L. H.

GREENSTEIN, J. P. [Nat. Cancer Inst., Bethesda, Md.] TITRATION OF THE LIVER CATALASE ACTIVITY OF NORMAL AND OF TUMOR-BEARING RATS AND MICE. J. Nat. Cancer Inst., 2:525-530. 1942.

Heretofore data on the liver catalase activity of normal and of tumor-bearing rats and mice have been obtained by the volumetric method. This method cannot conveniently be used to measure activities over a wide range of varying enzyme and substrate concentrations. The titration method, which is free from this disadvantage, follows the rate of disappearance of the hydrogen peroxide by the use of either permanganate or hydriodic acid. Descriptions of the two methods are given.

Three tables give results obtained by the titration method, of determinations of the liver catalase activity of normal rats and of rats bearing the transplanted hepatic tumor 31 and the transplanted Jensen sarcoma, as well as of normal mice and of mice bearing the transplanted sarcoma 37. These activities are expressed as ratios (under identical conditions) of k, the monomolecular reaction constant for each tissue. The ratios are equivalent to ratios of *Kat. f.* The liver catalase activity ratio of tumor-bearing to normal rats was 1:3–4 and of tumor-bearing to normal mice was 1:5. These values, while lower than those obtained by the volumetric method, are consistent with them in the finding of a much lower liver catalase activity in tumor-bearing as compared with normal animals.—F. L. H.

WHITE, F. P., and WHITE, J. [Nat. Cancer Inst., Bethesda, Md.] EFFECT OF A LOW CYSTINE DIET ON THE GROWTH OF VARIOUS STRAINS OF MICE. J. Nat. Cancer Inst., 2:449-450. 1942.

A diet was devised in which the cystine level could be readily controlled. This diet could be used over prolonged periods of time with various types of mice. The percentage composition of the low cystine diet and of a cystine-supplemented diet is given, the difference between the two being the replacement of 0.5% starch by 0.5% *l*-cystine in the high cystine diet. The two diets were tested on mice of both sexes of the C3H, ABC, Bagg albino,

C, A, dba (subline 212), I, and AK strains; all showed essentially the same type of response.

Mice fed on the low cystine diet for 12 months or longer showed no structural changes in organs or tissues but had a tendency toward rough coats and slow breeding. No permanent stunting occurred, as normal growth was resumed immediately and rapidly on addition of cystine to the diet. Mice were usually placed on the diets at 4 weeks of age. If started at 8 weeks they showed a tendency toward a slight loss of weight during the first week, but after that maintained their weight or gained slightly.—F. L. H.

WOODARD, H. Q., and KENNEY, J. M. [Memorial Hosp., New York, N. Y.] THE RELATION OF PHOSPHATASE ACTIVITY IN BONE TUMORS TO THE DEPOSITION OF RADIOACTIVE PHOSPHORUS. Am. J. Roentgenol., 47: 227-239. 1942.

Nine patients with osteogenic sarcoma, 4 with non-osteogenic malignant tumors, and 2 without tumors were studied in an attempt to correlate the phosphatase activity of tissue with actual phosphorus metabolism. Through the use of radioactive phosphorus as a tracer, it was found that phosphorus deposition in normal and hyperplastic bone was a function of the alkaline phosphatase activity of the tissue but this rule did not hold in all cases of osteogenic sarcoma. The osteogenic sarcomas which failed to store phosphorus in proportion to their high phosphatase content were generally the highly malignant tumors. Failure of the tumors to store phosphorus would constitute a limitation of the effectiveness of radioactive phosphorus in the treatment of osteogenic sarcoma.—C. E.D.

Transplantation

CLOUDMAN, A. M. [Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine] REACTIONS OF HYBRID AND PARABIONTIC PSEUDO-HYBRID MICE TO INOCULATIONS OF TUMOR C198. Cancer Research, 3:47-52. 1943.

More than 1,000 pure stock leaden mice were used as hosts in 55 successive transplant generations and all were positive to inoculation with the hepatic neoplasm C198 which arose spontaneously in this strain. Other stocks were negative after the usual methods of transplantation. No growths were obtained in 118 mice of the inbred strain C57 black. F_1 hybrids between the leaden and black C57 strains were completely susceptible to inoculation with grafts from the later transplant generations of C198.

A study was made of tumor inoculations following parabiosis. In 23 pairs of parabionts within the leaden strain, one mouse only was inoculated. In every case both members of the pair developed tumors in the liver. Black mice from the nonsusceptible C57 stock were united to susceptible leaden mice and, after healing was complete, inoculated with tumor C198. In 30 of 34 pairs the black mouse developed either subcutaneous or internal tumors. The tumor also passed from the black mouse into the leaden member of the pair.

The term "pseudo-hybrids" was employed to describe pure stock mice that showed a change from nonsusceptibility to susceptibility to C198 following parabiosis with susceptible mice. The altered pure stock mice react somewhat like hybrids between the stocks.—Authors' abstract.

RUSS, S., and SCOTT, G. M. [Barnato Joel Labs., Middlesex Hosp., London, England] EXPERIMENTS SHOWING THE INFLUENCE OF ONE GROWING TUMOUR UPON ANOTHER. Brit. J. Exper. Path., 23:127-133. 1942.

The growth of the Jensen rat sarcoma in one flank of an animal was accelerated by inoculation of another graft in the opposite flank, when the second graft was derived from a tumor growing much more rapidly than the original graft. The reverse effect was obtained by inoculating a graft growing much more slowly than the tumor already growing from the first implantation. In consistent experiments with adequate numbers, the tumors in the rats given the stimulating graft in the opposite flank eventually became twice the average volume of the controls; the tumors of the rats treated with the slowly growing grafts on the opposite flank were only one-third of the volume of the controls. Similar stimulation of Jensen sarcoma tissue, which had been given a sublethal dose of x-rays, was shown after the inoculation of a graft of rapidly growing normal tumor. Conversely, the inoculation of a graft of Jensen sarcoma, which had been given a sublethal dose of x-rays *in vitro* and was growing slowly, had an inhibiting effect on a normal tumor already growing in the rat, the average volume being only one-half that of the controls.—A. H.

Miscellaneous

SELBIE, F. R. [Bland-Sutton Inst. of Path., Middlesex Hosp., London, England] CARCINOMATOUS TRANSFORMATION IN A TRANSPLANTABLE RAT FIBROADENOMA. Brit. J. Exper. Path., 23:61-68. 1942.

In a transplantable rat fibroadenoma (obtained from a spontaneous tumor in 1934) a substrain has arisen in which 5 tumors in 3 successive passages have become manifestly carcinomatous at long intervals after inoculation. It is suggested that these changes can be ascribed to an increased proliferative capacity of the glandular tissue acquired by the parent tumor of the substrain. By its increased powers of proliferation the epithelium can overcome the restraint of the fibrous component of the tumor and can thus make manifest its intrinsic malignancy. Changes had previously been described in the fibrous component of this tumor (Brit. J. Exper. Path., **22**:156. 1941; abstract in Cancer Research, **1**:909. 1941) leading to the appearance of new sarcomatous tumors.—A. H.

Comparative Oncology

GREENE, H. S. N., and BROWN, W. H. [Rockefeller Inst. for Med. Research, Princeton, N. J., and Yale Univ. Sch. of Med., New Haven, Conn.] A TRANSPLANTABLE SQUAMOUS CELL CARCINOMA IN THE RABBIT. Cancer Research, 3:53-64. 1943.

The course of a squamous cell carcinoma in the skin of a rabbit was followed from inception of the growth to the death of the animal, and clinical observations were supplemented by frequent biopsies and attempts to transfer the tumor. Histologically the primary tumor conformed in part to a type known in man as spindle cell epidermoid carcinoma, but the metastases were all squamous cell in character. The tumor was successfully transplanted and the histological character of the subsequent growths varied according to locality.—Authors' abstract.

Clinical and Pathological Reports

Skin and Subcutaneous Tissues

CAMPBELL, D. A. [Univ. of Mich. Med. Sch., Ann Arbor, Mich.] CARCINOMA OF THE UPPER EXTREMITY. Arch. Surg., 43:803-810. 1941.

In the series of 36 cases reported in this article the average age incidence was 61.6 years. There was a ratio of 8 men to 1 woman. About 64% of the lesions occurred on the dorsum of the extremities. Metastases occurred in 44.4% of the cases. The average duration of the disease was 29½ months. It is noted that these lesions of the extremities are too often overlooked and undertreated. Wide surgical excision, with regional lymph node irradiation, has yielded good results.—R. C. R.

HOLTZMANN, I. N., and BOLKER, H. [Brooklyn Cancer Inst., Brooklyn, N. Y.] BASAL CELL EPITHELIOMA; A CONCEPT OF ITS HISTOGENESIS. Am. J. Roentgenol., 47:463-466. 1942.

The origin of all primary epithelial tumors of the skin is traced to basal cells and a classification on this basis is presented. The failure of basal cell carcinomas to metastasize is explained on the assumption that the normal basal cells form a syncytium which is maintained in the tumors. Ulceration is attributed to invasion of lymphatic vessels by the tumor with compression of neighboring blood vessels and regional ischemia.—C. E. D.

SUTTON, R. L., Jr. [Univ. of Kansas Sch. of Med., Kansas City, Kan.] EPITHELIOMA OF THE SKIN. MANNER OF GROWTH: HISTOLOGIC STUDY OF WHOLE SECTIONS. Arch. Dermatol. & Syph., 46:1-39. 1942.

"Progressive cancerization" at the edges of cutaneous epitheliomas is judged a misinterpretation of histologic data, which reveal abnormal cells only in juxtaposition to normal ones, undermining, replacing, and engulfing them. No evidence of conversion of normal cells into cancerous cells is found in diagnosable epitheliomas of the human skin. Malignancy and invasiveness are presented as phenomena intimately concerned with cohesion of cancer cells and the rate of their growth. "Precancerosis" is discussed and its absence of biologic meaning emphasized. Evidence is presented that, clinically and histologically, each spontaneous epithelioma of the human skin behaves like a colony of cells of a new kind—possibly the homogenous progeny of one mutant cell.—H. G. W.

Oral Cavity and Upper Respiratory Tract

DUNNE, R. E. [Hartford, Conn.] CANCER OF THE LIP. Connecticut M. J., 6:175-176. 1942.

Eighty per cent of cancers of the lip are curable if the lesion is recognized early enough and treated correctly. Indications for surgery are a large primary tumor and metastases to the nodes. Smaller lesions should be treated by irradiation. A prophylactic neck dissection is not advised.—R. C. R.

HOWES, W. E., and LaROSA, F. J. [Brooklyn Cancer Inst., Brooklyn, N. Y.] CARCINOMA OF THE LOWER LIP: INTERVAL STATISTICAL SURVEY OF THE END RESULTS IN ALL CASES TREATED AT THE BROOKLYN CANCER INSTITUTE, 1930 TO 1939 INCLUSIVE. Am. J. Roentgenol., 47:39-49. 1942.

Seventy-four cases of proved carcinoma of the lower lip have been followed at the Brooklyn Cancer Institute for periods of 6 months to 6½ years. All but one of these tumors occurred in males and all were diagnosed as squamous cell carcinoma with the exception of one basal cell carcinoma. The lesions were treated by surgery, radium, or roentgen rays. At the end of an average follow-up period of over two years, the authors consider 59 cases to have been successfully treated, although this figure includes 3 patients who died from intercurrent disease and 17 who were lost track of.—C. E. D.

KEYES, E. L. [Washington Univ. Med. Sch., St. Louis, Mo.] CHRONIC ULCERATIVE LESIONS OF THE MOUTH. INCIDENCE OF CANCER IN 2,077 CASES AT BARNARD HOSPITAL. Am. J. Surg., 56:70-78. 1942.

Of 2,077 chronic ulcerative lesions of the mouth examined microscopically at the Barnard Hospital, no less than three-fourths were malignant. Every physician or dentist should appreciate that when a patient has an ulcer of the mouth that has lasted more than a week the chances are three to one that it is malignant. Of the 1,561 malignant lesions, 1,523 were squamous cell carcinoma, 28 were sarcoma, 8 adenocarcinoma, and 2 basal cell carcinoma. Squamous cell carcinoma of the mouth is chiefly a disease of men—91.1% of 406 patients were male. Lip carcinoma is even more a disease of the male—only 3 of 787 instances of squamous cell cancer of the lip occurred in women. Cancer is rare in a clean mouth; it is usually found associated with foul teeth and with excessive use of tobacco. Of 124 cases of cancer of the lip, in but 4 did it arise from the upper lip. The 1,523 cancers of the mouth were located as follows: lip, 787; buccal mucosa, 564; tongue, 172. There were also 180 benign neoplasms in the 2,077 cases of mouth lesions, of which 63 were epulis and fibromas, 38 angiomas, 31 polyps or papillomas, and 26 cysts.—H. G. W.

MacCOMB, W. S., and MARTIN, H. E. [Memorial Hosp., New York, N. Y.] CANCER OF THE NASAL CAVITY. Am. J. Roentgenol., 47:11-23. 1942.

Sixty-five patients with malignant tumor of the nasal cavity were seen at the Memorial Hospital from 1928 to 1940. This group made up about 0.7% of all persons admitted for cancer of the upper respiratory and alimentary tracts. Forty-one patients were male and 24 female. Their ages ranged from 5 to 84 years with an average of 55. Of the 65 tumors, 34 were classified as epidermoid carcinoma, 7 as transitional cell carcinoma, 4 as lympho-epithelioma, 2 as melanoma, 11 as adenocarcinoma, and 7 as sarcoma.

Only 19 patients developed metastases, the lymph nodes of the neck usually being involved. In 6 patients there were distant metastases. With the exception of one adenocarcinoma, all metastasizing tumors were epidermoid carcinoma. The most frequent cause of death was uncontrolled local disease complicated by hemorrhage or infection.

Radiosensitive tumors were treated with 4,000 to 5,000 r of roentgen rays while radioresistant growths were removed by radical operation whenever feasible. In 35 cases that were followed, 5 year cures were obtained in 4 instances of epidermoid carcinoma, 2 of lympho-epithelioma, and 1 each of adenocarcinoma and angio-

sarcoma, making a total of 8 cases or about 23%. No patient with metastases survived.—C. E. D.

SCHUSTER, F. P., and SCHUSTER, S. A. [El Paso, Tex.] POSTNASAL FIBROMA. Texas State J. Med., 37:782-784. 1942.

Two instances of successful excision of large postnasal fibromas are recorded.—M. J. E.

Bone and Bone Marrow

BADGLEY, C. E., and BATTS, M., Jr. [Univ. of Michigan Med. Sch., Ann Arbor, Mich.] OSTEOGENIC SARCOMA. AN ANALYSIS OF EIGHTY CASES. Arch. Surg., 43:541-550. 1941.

Eighty patients with unquestionable osteogenic sarcoma were admitted to the University Hospital between 1925 and 1938. Of these, 64% were men and 36% women. The ages varied between 8 and 72 years, with an average at 29 years and half of the patients in the second decade of life. Thirteen per cent of the patients had a tumor-positive family history. Thirty-six per cent gave a history of preceding trauma of various grades. The average duration of symptoms from the onset to the time of admission was 13 months. The outstanding symptoms were pain and swelling. A palpable tumor was present in 91% of the cases. These tumors showed a definite predilection for the ends of the long bones, 50% of them being in the region of the knee. Classification of the neoplasms with respect to five year survivals showed that sclerosing osteogenic sarcoma and secondary chondrosarcoma were the least malignant of the group. The treatment of choice was radical amputation without preoperative irradiation therapy. A follow-up of this series revealed 78% mortality to date. The average survival period was 41 months. The prognosis appeared to be better if the lesions occurred after the twentieth year.—R. C. R.

CAUBLE, W. G. [Kansas City Gen. Hosp., Kansas City, Mo.] OSTEOGENIC SARCOMA OF THE STERNUM. SURGICAL REMOVAL FOLLOWED BY ROENTGEN RAY THERAPY. REPORT OF A CASE. J. Missouri M. A., 38:408-410. 1941.

A sarcoma of the sternum was treated by combined surgical and roentgen therapy with apparently excellent results. Six months later, roentgen examination of the chest showed evidence of metastases.—M. J. E.

DONHAUSER, J. L., and de ROUVILLE, W. H. [Albany Med. Coll., Albany, N. Y.] MULTIPLE MYELOMA, WITH SPECIAL REFERENCE TO SOFT TISSUE METASTASIS. Arch. Surg., 43:946-1020. 1941.

A discussion of 5 cases of the disease, including blood chemistry, urinary and physical findings, symptoms, pathology, differential diagnosis, and roentgenology, is presented. It is emphasized that all patients in the sixth decade of life presenting pain in the back, in the absence of injuries or history of injuries, should be watched carefully in an attempt to rule out multiple myeloma. The importance of frequent blood examinations is stressed.—R. C. R.

HAFSTRÖM, T. [St. Erikkrankenhauses, Stockholm, Sweden] DAS VERHALTEN DER TAKATA-REAKTION BEIM MYELOM. [TAKATA REACTION IN CASES OF MYELOMA.] Acta Med. Scandinav., 106:61-69. 1941.

A positive Takata reaction is found in the blood in cases of multiple myeloma, when there is a coexisting increase in the globulin content. With normal blood protein values, the reaction is negative. Two case reports illustrate these generalizations.—M. J. E.

Leukemia, Lymphosarcoma, Hodgkin's Disease

BURGER, R. E., and LEHMAN, E. P. [Univ. of Virginia Hosp., Charlottesville, Va.] HODGKIN'S DISEASE. REVIEW OF FIFTY-FOUR CASES. Arch. Surg., 43:839-849. 1941.

An analysis of 54 histologically proved cases of Hodgkin's disease is presented. Of these, 83.3% were in white persons as compared with 16.7% in negroes. There was a predominance of males (59.4% as against 40.6% of females). The ages varied between 4 and 70 years, with the greatest number of patients in the third decade of life. No predisposing factors were demonstrable. The chief complaint in 57.4% of the cases was swelling of the cervical lymph nodes, and in 53.7% of the cases this was the region first involved. The most common symptoms prior to hospitalization were weakness, loss of weight, cough, and fatigue. The average duration of life in 41 patients of the group was 26 months from the onset of the symptoms. Radiation, blood transfusions, and general therapy increased the survival period nearly three times.—R. C. R.

de JONG, J. J. (Krankenhauses d. Reichsuniversität, Leiden, Holland] EINE BESONDERE FORM DER LEUKÄMIE. [AN UNUSUAL FORM OF LEUKEMIA.] Acta Med. Scandinav., 108:251-260. 1941.

A description of an atypical form of chronic myeloid leukemia is based upon records of 4 cases. Clinically, with the exception of the benign, protracted course, the disease does not differ essentially from the ordinary forms of leukemia. Hemorrhagic manifestations are common. However, instead of enormous increases in the number of circulating leucocytes, counts of 20,000 to 40,000 are the usual finding. Although immature forms of leucocytes are rarely encountered peripherally, obvious leukemic alterations are evident in the sternal marrow. The erythrocytes are not specifically altered. Corroborative proof of the true nature of the process is the presence of leukemic infiltrations in the spleen, observed in a patient who died of a complicating gastric hemorrhage. Two patients treated symptomatically continue in satisfactory condition; the status of the fourth is unknown.—M. J. E.